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MORPHOGENETIC AND ULTRASTRUCTURAL INVESTIGATION IN THE HYPOTRICHIDA (CILIOPHORA, PROTOZOA): IMPLICATIONS IN CILIATE EVOLUTION

BARRY JAMES WICKLOW

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University of New Hampshire PH.D. 1982

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BY

Barry J. Wicklow
B.S., Central Connecticut State College, 1972
M.S., University of New Hampshire, 1975

DISSERTATION

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

Doctor of Philosophy
in
Zoology

May, 1982

This dissertation has been examined and approved.

Dissertation Director, Arthur C. Borror, Professor of Zoology, University of New Hampshire

John O. Corliss, Professor of Zoology
University of Maryland

Robert A. Croker, Professor of Zoology
University of New Hampshire

Larry G. Harris, Associate Professor of Zoology
University of New Hampshire

Yun-Tzu Kiang, Associate Professor of Plant Science and Genetics, University of New Hampshire

Charles W. Walker, Associate Professor of Zoology
University of New Hampshire

5 MAY 82
Date

To My Family

We need the tonic of wildness--to wade sometimes in marshes where the bittern and the meadow-hen lurk, and hear the booming of the snipe; to smell the whispering sedge where only some wilder and more solitary fowl builds her nest, and the mink crawls with its belly close to the ground. At the same time that we are earnest to explore and learn all things, we require that all things be mysterious and unexplorable, that land and sea be infinitely wild, unsurveyed and unfathomed by us because unfathomable. We can never have enough of Nature. We must be refreshed by the sight of inexhaustible vigor, vast and Titanic features, the seacoast with its wrecks, the wilderness with its living and its decaying trees, the thundercloud, and the rain which lasts three weeks and produces freshets. We need to witness our own limits transgressed, and some life pasturing freely where we never wander.

Henry David Thoreau

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I wish to express my deep gratitude to Dr. Arthur C. Borror, Professor of Zoology, University of New Hampshire for both his guidance and supervision during the course of this study, as well as his creativity and scientific expertise which made him a superlative model during my investigations of the natural world. I thank the members of my advisory committee for their review of and comments on this thesis, and Dr. Marcel Lavoie for his kind assistance in the writing and reading of French. I am grateful to the staff of the Jackson Estuarine Laboratory, especially Dr. Arthur C. Mathieson and Elenor Tveter Gallagher for their generous support. I am indebted to Eileen Wong for her excellent typing of this thesis and to Ted Donn and Chuck Towle for their help in collecting marine sand samples. Thanks to my fellow students - Luther Black, Patty Burke, Don Culberson, Linda Deegan, Wendy Lull, and many others - who by their friendship and comradery have added so much to my graduate studies. I extend my sincere gratitude to Dr. William J. Jahoda, Professor Emeritus, scientist, educator, and naturalist, whose wisdom and inspiration led me on my present path.

I gratefully acknowledge financial support of this research by Central University Research Fund grant numbers S75 and S96, University of New Hampshire.

I express my deepest feelings of appreciation to my wife Patricia and to my son Nathan for the understanding, encouragement, and joy they have given me during the course of this study. Thank you.

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ABSTRACT

MORPHOGENETIC AND ULTRASTRUCTURAL INVESTIGATION
IN THE HYPOTRICHIDA (CILIOPHORA, PROTOZOA):
IMPLICATIONS IN CILIATE EVOLUTION

by

BARRY J. WICKLOW

University of New Hampshire, May, 1982

Hypotrichs represent a highly diverse species ensemble displaying a wide range of form and function that reflects extensive radiation in a wide variety of habitats. Because of their constancy through time, particular habitats such as marine sands harbor forms that may have persisted with little change for hundreds of millions of years and, hence, may hold clues to help unravel mysteries of ciliate evolution. Using light optical microscopy and both scanning and transmission electron microscopy, I examine cortical morphogenesis through cell division and interphase ultrastructure of several species of marine ciliates. Using this information I propose phylogenetic relationships within the Hypotrichida, suggest homologies between hypotrichs and other ciliates, discuss the adaptive significance of ciliate microtubular organelles, and explore the evolution of cellular complexity in general.

Thigmokeronopsis and the Urostylina

Thigmokeronopsis jahodai n.gen., n.sp. is a benthic estuarine ciliate. The morphogenetic pattern is of the urostyline type: frontal

ciliature develops from a longitudinal series of oblique streaks. I interpret the postmembranellar fiber in Thigmokeronopsis as homologous with the postciliodesmata of karyorelectid and heterotrichine ciliates. I consider cirri and membranelles as oligomerized organellar complexes having evolved by amplification (polymerization) of kinetosomal pairs with concommitant reduction of microtubular derivatives and a general decrease in the number of organellar complexes per cell as more complex hypotrichs evolved.

Discocephalus and the Discocephalina (n.subord.)

I described morphogenesis, ultrastructure, and polymorphism in Discocephalus ehrenbergi as well as structure and morphogenesis in Psammocephalus borrori (n.gen., n.sp.), Psammocephalus faurei (n.comb.), and Amphisiella marioni. The developmental pattern of D. ehrenbergi is divergent from that of other hypotrichs. Unique ultrastructural specializations (e.g. peristomial cirrus, dorsal bristle complex, microtubular spines) characterize this genus. Species of the genus Psammocephalus are structurally and developmentally similar to Discocephalus but different from Amphisiella and serve as intermediates in an evolutionary series that demonstrates a trend in the Discocephalina toward increased cytoskeletal complexity, cirral reduction and hypertrophy, and ovoid cell shape.

Certesia and the Euplotina

Postciliary microtubules from membranellar kinetosomes line the buccal cavity and separate parallel arrays of pharyngeal discs. Alveolar plates lie within alveolar membranes; lasiosomes are present. During morphogenesis microtubular bundles appear beside (later attached

to) developing frontal anlagen; they disappear after cirri are in final interphase locations. These microtubules may be involved in mediating placement of cortical structures during morphogenesis. Although possessing unique characters, Certesia shares a close phylogenetic relationship with Euplotes.

Epiclintes and the Epiclintina (n.subord.)

Morphogenesis is based on 4 kinds of primordia: oral, caudal (unique to Epiclintes), frontal (in the opisthe only), and somatic. The morphogenetic pattern of Epiclintes is divergent from other hypotrichs. Specializations of the dorsal bristle complex and cortex are unique to Epiclintes. The above information falsifies hypotheses that include Epiclintes in present hypotrich groups.

EVOLUTION WITHIN THE ORDER HYPOTRICHIDA (CILIOPHORA, PROTOZOA): ULTRASTRUCTURE AND MORPHOGENESIS OF *THIGMOKERONOPSIS JAHODAI* (N.GEN., N.SP.); PHYLOGENY IN THE UROSTYLINA (JANKOWSKI, 1979)

CHAPTER I

EVOLUTION WITHIN THE ORDER HYPOTRICHIDA (CILIOPHORA, PROTOZOA): ULTRASTRUCTURE AND MORPHOGENESIS OF THIGMOKERONOPSIS JAHODAI (N.GEN., N.SP.); PHYLOGENY IN THE UROSTYLINA (JANKOWSKI, 1979)

Introduction

Hypotrichs, with their high level of structural and developmental complexity, represent an evolutionary peak among the diverse assemblage of species composing the phylum Ciliophora. The presence of organellar complexes: paramembranelles, locomotory cirri (particularly frontal cirri), and associated fibrillar systems characterize most of the 500 known species. Hypotrichs display a wide range of form and function reflecting extensive radiation in a variety of habitats. Numerous adaptive strategies have been successfully exploited leading to a highly diverse species ensemble. Planktonic, interstitial, thigmotactic, sessile, tubicolous and stalked species exist; they can be coiled, contractile or rigid; both encysting and budding forms are known; most are free-living, others endo- or ectocommensal; they can be cephalized or vermiform, concave and reniform, oval, flattened or fusiform. This diversity of form and function should be reflected in current systematic schemes.

FAURÉ-FREMIET, in 1961, proposed 2 suborders within the Hypotrichida: the Stichotrichina and the Sporadotrichina. This division has been accepted in the classification schemes of JANKOWSKI (1967), PUYTORAC et. al. (1974), CORLISS (1977), and LEVINE et. al. (1980). Such a division was opposed by BORROR (1972) due to the high diver-

sity within the overlapping ontogenetic patterns between, the two suborders.

FAURÉ-FREMIET included 8 families within his 2 hypotrich suborders in 1961; CORLISS, in 1979, recognized 11 families: Aspidiscidae, Euplotidae, Gastrocirridae, Holostichidae, Keronidae, Kiitrichidae, Oxytrichidae, Psilotrichidae, Spirofilidae, Strongylidiidae, and Urostylidae. BORROR (1979) transferred the genus Holosticha to the family Urostylidae, thereby dropping the name Holostichidae as a junior synonym. Also in 1979, TUFFRAU described an additional family, the Kahliellidae. JANKOWSKI (1979) in his revision of the order suggests new names for many previously described taxa, elevates many genera to family status and proposes superfamilial and subordinal changes that he deems acceptable.

Recently both ultrastructural and morphogenetic information have been used to advantage in ciliate systematics and phylogeny. LYNN (1976), and GIERRASSIMOVA and SERAVIN (1976) have championed the use of somatic kinetal ultrastructure as highly conservative data in character phylogeny. PUYTORAC et. al. (1976) and SMALL (1976) have relied heavily on the ultrastructure of the oral apparatus in their systematic studies. BORROR (1972, 1979a, 1979b) and CORLISS (1968) have argued for the use of morphogenetic data in constructing natural ciliate phylogenies. Clearly a combined approach is warranted.

Ideas of DOGIEL (1929), later re-emphasized by POLJANSKI and RAIKOV (1976), suggest the hypothesis that an interaction of polymerization and oligomerization of homologous structures has played a major role in fashioning organellar complexes during ciliate evolution. Use of both ultrastructure and the orderly process of development

as "lenses" may allow testing of such a hypothesis and culminate in a more lucid view of hypotrich phylogeny.

In the present study I examine the ultrastructure and morphogenesis of Thigmokeronopsis jahodai n.g., n.sp., propose a phylogeny of Urostyline hypotrichs and explore the possible course of evolution of hypotrich organellar complexes in general.

Materials and Methods

I isolated Thigmokeronopsis jahodai from surface sediments (mostly gravel and crushed shell) of Great Bay near Jackson Estuarine Laboratory, Adams Point, New Hampshire. Populations grow in culture on the diatom Phaeodactylum in 30 °/oo seawater at 16°C.

I stained cells with BORROR's nigrosin-butanol method (1979b) or a modification of TUFFRAU's protargol technique (1967): after fixation in PERENYI's solution (with 0.3 g $NaCl_2$ per 10 ml added to adjust osmolarity) cells are dehydrated, affixed to albumenized coverslips and covered with a thin layer of collodion before proceeding to bleaching and staining (HAMMERSMITH, pers. comm.).

I fixed cells for S.E.M. in a 1:1 mixture of 2% O_sO_4 in 30 °/oo seawater and 3% glutaraldehyde in 30 °/oo seawater for 30 minutes, followed by dehydration to 100% ethanol. Then I transferred a concentrated drop of cells, while observing with a dissecting microscope, to a coverslip coated with a thin layer of albumen. Before the cells air dried, I flooded the coverslip slowly with additional 100% ethanol, changed this fluid by adding drops of formal-alcohol (a 1:1 mixture of 8% formalin and 95% ethanol) to one side of the coverslip until the evaporating ethanol was completely replaced. This reduces turbulance produced when adding formal-alcohol directly to 100% ethanol. After 2 minutes, I transferred coverslips to a Coplin jar containing formal-alcohol for 30 minutes.

Specimens adhere immediately to the coverslips upon denaturation of the protein layer by ethanol. The formal-alcohol mixture completes the

albumen fixation, thus securing the cells to the coverslip. After the cells are critical point dried, they are sputter coated with heavy metals, attached to stubs and viewed in an AMR 1000 S.E.M. while still on coverslips.

This method facilitates handling of small specimens (I have also processed Tardigrades and Hydra using this technique) normally difficult to prepare for S.E.M. Furthermore, I can prepare them for T.E.M. even after they are prepared for S.E.M. (SPINDLER, 1978). This capability allows analysis of cortical features or patterns with subsequent study of subcortical structures in the same cell.

I fixed cells for T.E.M. using the same O_sO_4-glutaraldehyde mixture described above, washed them 3 times in either 0.1m PO_4 buffer adjusted to 900 milliosmols with sucrose or 30 ‰ seawater and dehydrated them in a graded series of ETOH. I infiltrated embedded specimens using SPURR's standard medium in pyrex watch glasses. Polymerization took place at 70°C overnight. The polymerized plastic separated from the watchglass upon immersion in liquid nitrogen. As I can view the cells clearly with a compound microscope, I then cut them out, orient them for the desired cutting plane, affix them to blank blocks and section them using a Reichert OM3 ultra-microtome.

I picked up sections on formvar coated grids, stained with uranyl acetate (30 min.) and lead citrate (15 min.), then viewed them in a JEOL 100S T.E.M.

Results

Interphase Structure

The general cortical anatomy of Thigmokeronopsis jahodai is illustrated in a camera lucida drawing of a protargol stained cell (fig. 1) and in S.E.M. (figs. 2-6); all references to the cell will be relative to the cell's left or right: in ventral aspect, the cell's left corresponds to the reader's right. Cell length ranges from 180-240 m (x=208 m, N=20), cell width ranges from 55-85 m (x=67 m, N=20).

The ciliate is supple and slightly contractile; a 200 m individual can stretch to 400 m when feeding actively. When subjected to a current (such as water forced from a pipette) the cell adheres firmly to the substrate. Adhesion is accomplished by a thigmotactic ciliary field (described below). Irregular groups of yellow-green granules are scattered subcortically through the cytoplasm. These structures are more numerous on the dorsal surface. A buccal cavity plunges 50 m into the cytoplasm from the posterior buccal overture. Food vacuoles contain numerous and sundry diatoms. Over 100 oval macronuclei are dispersed throughout the cytoplasm.

Hypotrichs possess 3 kinds of ciliature: buccal, frontal and somatic (BORROR, 1979). Buccal ciliature in Thigmokeronopsis consists of 75 membranelles and a two part undulating membrane: an endoral membrane consisting of a single row of cilia and a paroral membrane consisting of multiple arrays of cilia.

Somatic ciliature includes 4 rows of dorsal bristles (fig. 3); each bristle complex contains 2 kinetosomes - the anterior is ciliated, the posterior is not. One left marginal and one right marginal row of cirri

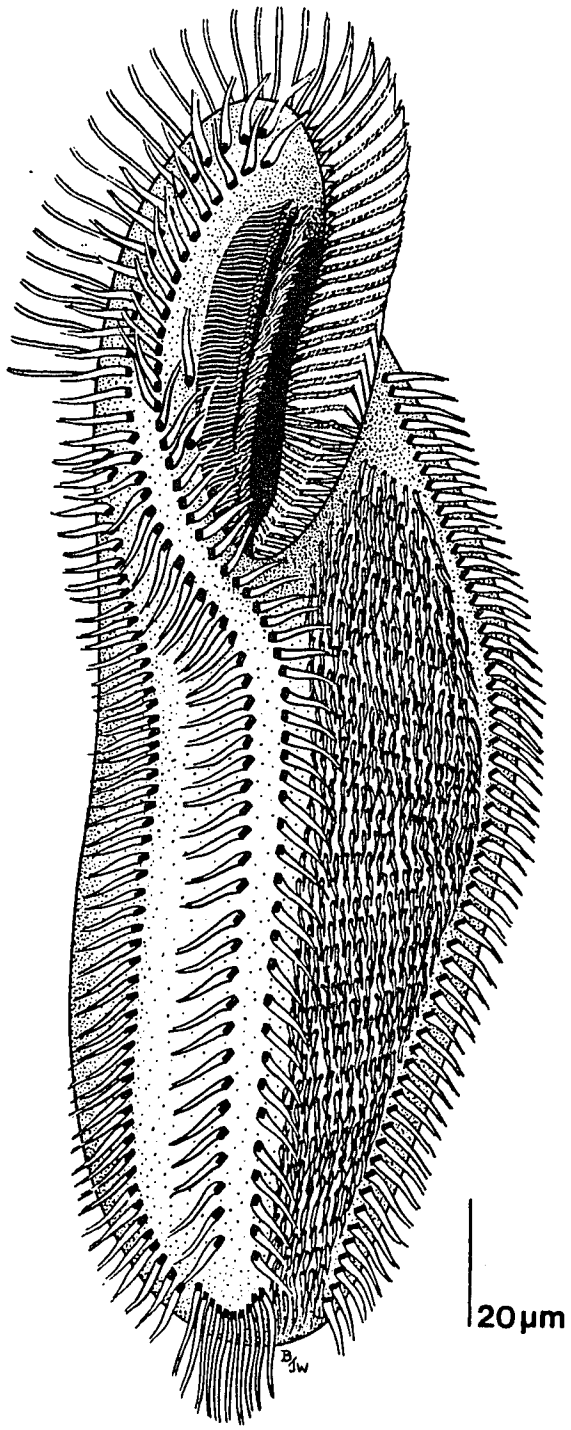
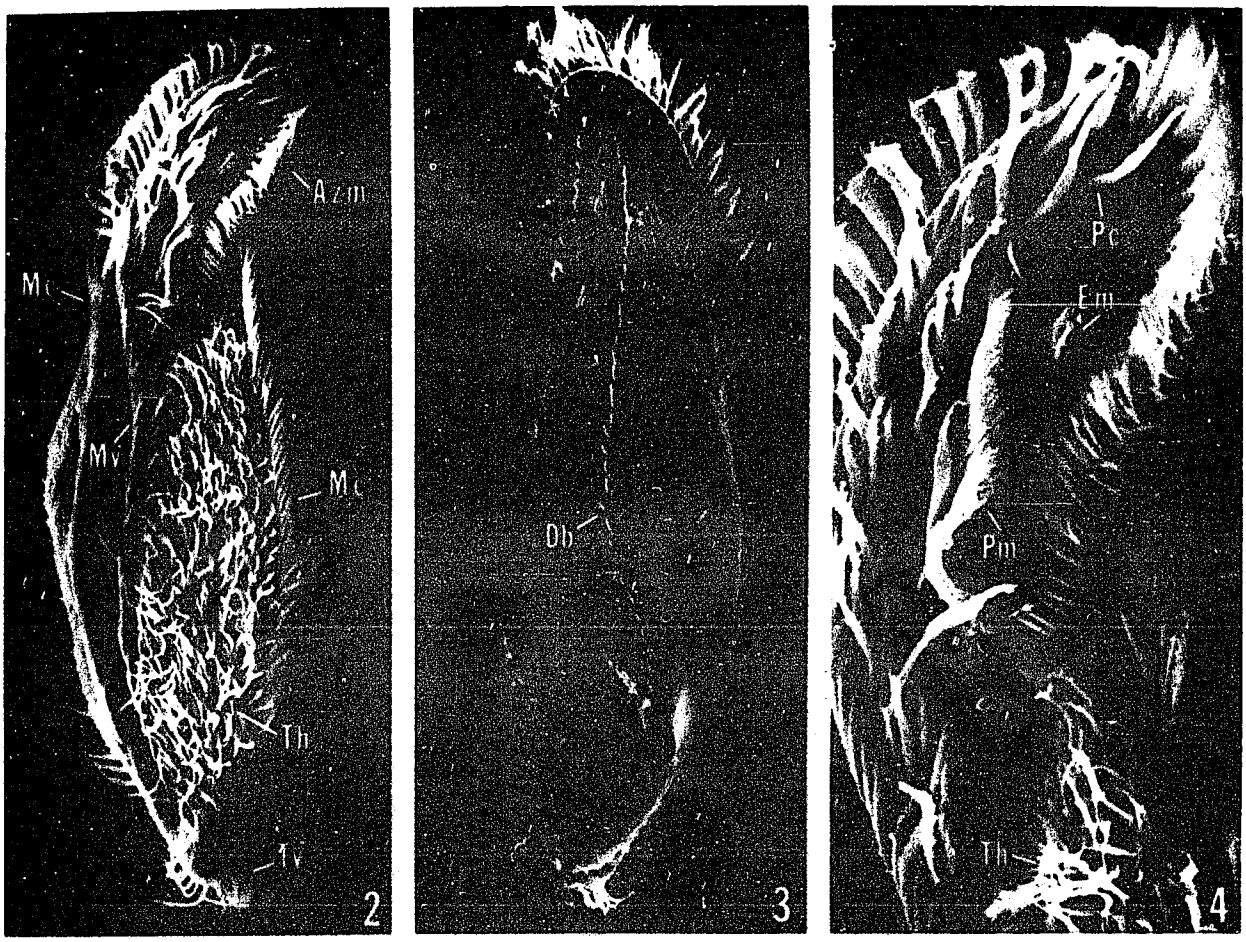


Fig. 1. Ink drawing of Thigmokeronopsis jahodai n.g., n.sp., based on a protargol stained specimen (ventral aspect).

Fig. 2, 3. Scanning electron micrographs of non-dividing cells. Ventral aspect (fig. 2): Azm - adoral zone of membranelles, Mc - marginal cirri, Mv - midventral cirri, Th - thigmotactic cirri, Tv - transverse cirri. Dorsal aspect (fig. 3): Db - dorsal bristles. (X 730, 700).

Fig. 4, 5, 6. Scanning electron micrographs depicting structures of the cells anterior ventral cortex. The adoral zone of membranelles (Azm) and endoral membrane (Em) descend into the buccal cavity; intermembranellar ridges (Imr) are visible between membranelles and a ribbed wall (Rw) supports the right side of the buccal cavity; a paroral membrane (Pm) lies within a shallow groove along the left buccal overture. Positions of frontal ciliature, including the paroral cirrus (Pc), malar cirri (Ma), migratory cirri (Mg), midventral cirri (Mv), and thigmotactic cirri (Th) are evident. (X 1 600, 2 640, 3 580).


Azm
Mv
Mv
Th
Tv
2
Db
3
Pc
Em
Pm
Th
4
Mg
Ma
Pm
Mv
5
Rw
Inr
Pm
Azm
Ma
6

are also present.

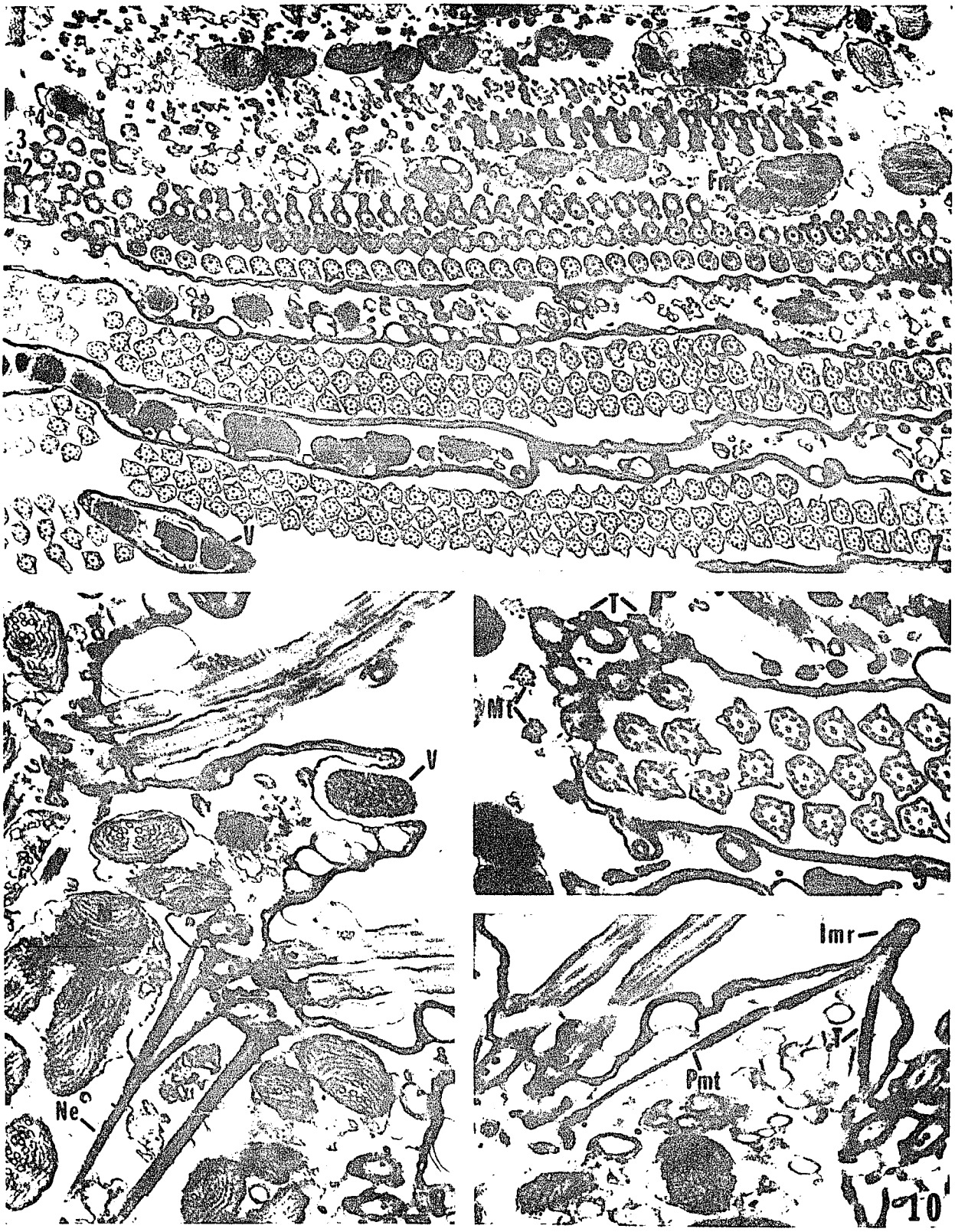
Frontal ciliature comprises paroral (buccal), malar, midventral, transverse, migratory, and thigmotactic cirri (malar and migratory cirri are defined below). A single paroral cirrus lies just anterior to the buccal cavity and 2 malar cirri lie adjacent to the paroral membrane (figs. 4-6). Just right of the paroral cirrus and curving to the posterior end of the cell are 2 rows of ~54 midventral cirri; a U-shaped group of transverse cirri subtends this series (fig. 1, 2). Two migratory cirri are present to the right of the midventral row, just posterior to the distal collar membranelles (figs. 1, 5). A cirral field, located between the midventral and left marginal cirral rows, consisting of a series of ~42 transverse rows, compose the thigmotactic ciliature (figs. 1, 2).

Ultrastructure

Buccal apparatus. The membranelles are paramembranelles (as defined by PUYTORAC and GRAIN, 1976) and each comprises 4 rows of kinetosomes: the first (posteriormost) and second are of equal length and are the longest rows (~40 kinetosomes), the third is shorter (~25 kinetosomes) and the fourth row is the shortest with only 3-6 kinetosomes (fig. 7). In general, the membranelles in the middle lapel region have longer rows than membranelles in either proximal or distal regions. A series of microfibrillar units couch the proximal edge of both anterior and posterior rows of membranellar kinetosomes and extend toward the cell surface (fig. 7). Both inter- and intramembranellar fibrillar connections are present.

Nematodesmal microtubules originate near the proximal end of membranellar kinetosomes, plunging into the cytoplasm at a slight posterior

Fig. 7, 8, 9, 10. Transmission electron micrographs of the adoral membranelles. Each membranelle comprises 4 kinetosomal rows (fig. 7): row 1 (posteriormost) and row 2 are longest, row 3 is shorter and row 4 is shortest; fibrillar material (Fm) borders rows 1 and 4. Nematodesmal microtubules (Ne) extend from the base of membranellar kinetosomes (fig. 8). Transverse microtubules (T) of row 4 extend distally toward postciliary microtubules (Pmt) from row 1 of the adjacent membranelle; both unite within the intermembranellar ridge (Imr). Microtubules (Mt) extend from the left edge of the membranelles (fig. 9) possibly supporting the ribbed wall (fig. 6). Mitochondria (M) and vesicles (V) are arranged between membranelles. (X 13 900, 21 400, 31 200, 21 200).


3
2
1
Fm
Fm
V
V
Ne
Pmt
Imr
Pmt
10

angle (fig. 8). Groups of mitochondria are arranged between these fibers.

Transverse microtubular ribbons are associated with the third and fourth membranellar rows; these originate at the middle zone of the kinetosome and extend toward the cell surface within an intermembranellar ridge that separates each membranelle (fig. 9). Postciliary microtubular ribbons originate from the posterior border of the first kinetosomal row, run toward the cell surface within the intermembranellar ridge, meeting the transverse microtubules of the adjacent membranelle (fig. 10).

The postciliary ribbon then courses left along the intermembranellar ridge, joins postciliary ribbons from other kinetosomes within the row and unites with ribbons from other membranelles to form a postmembranellar fiber (fig. 11). This fiber runs posteriorly along the left border of the lapel membranelles and subtends the proximal membranelles (fig. 15).

To the right of each membranelle are found additional bundles of microtubules. These extend along the left wall of the buccal cavity and are probably the supporting elements of the ribbed wall observed in S.E.M. (figs. 6, 9).

Electron opaque vesicles are found near the cell surface bordering the membranelles and within the intermembranellar ridges (figs. 7, 8).. These osmophilic vesicles are presumed to be the yellow-green granules observed at the light microscope level. At times they appear to be expelled from the cell surface (fig. 8).

The single row of kinetosomes of the endoral membrane lies within the dorsal wall of the buccal cavity and continues toward the cytostome,

Fig. 11, 12, 13. Transmission electron micrographs of the oral apparatus. A postmembranellar fiber (Pmf) is formed by the union of postciliary microtubules from each membranelle (fig. 11); fibrillar connections (Fc) and nematodesmal microtubules (Ne) are evident. The endoral membrane (fig. 12) consists of a single row of kinetosomes (stichomonade) with transverse microtubules (T) on the left and postciliary microtubules (Pmt) on the right. The paroral membrane (fig. 13) comprises multiple arrays of kinetosomes in a longitudinal series (polystichomonade); transverse microtubules (T) extend from the left most kinetosome. Ant - anterior, Pos - posterior, V - vesicle. (X 16 500, 31 500, 31 500).


3
2
Fc
Me
Pmf
11
Ant
Pmt
T
V
Pos
12
V
T
Ant
Pos
13

Fig. 14, 15. Transmission electron micrographs of the postmembranellar fiber (Pmf) that subtends the adoral zone of membranelles (Azm) then extends along the paroral membrane (Pm, fig. 14). A portion of the endoral membrane (Em, fig. 15) is visible. (X 9 400, 3 000).

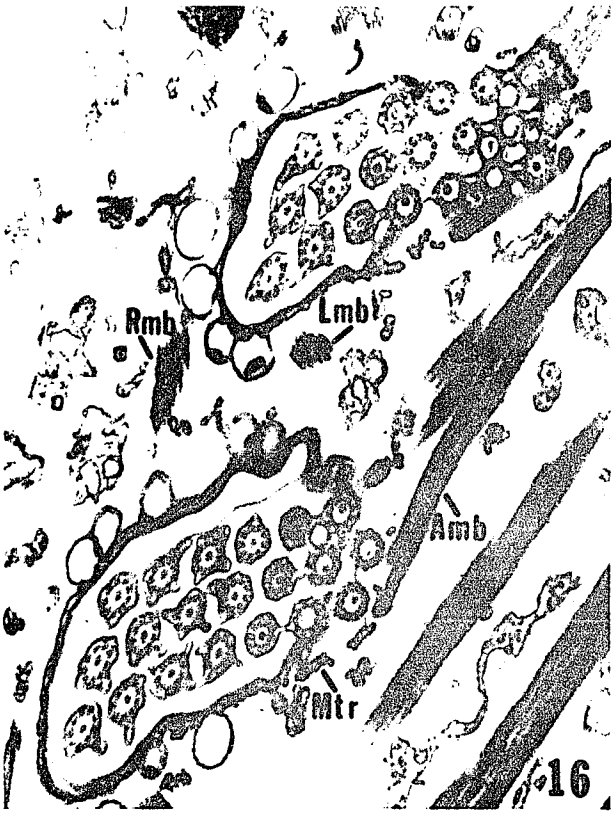

Pm
Pmf
14
Azm
Pmf
Em
15

well beyond the posterior limit of the paroral membrane and the proximal membranelles. The anterior end of the endoral membrane is oriented with the distal end of kinetosomes nearest the ventral surface of the cell; as the row descends into the buccal cavity, it rotates 180° along the longitudinal axis until the proximal end of each kinetosome is nearest the cell's ventral surface. This causes the proximal microtubules of each kinetosome to appear as a clockwise cartwheel. This rotation is not, however, reflected in the position of the postciliary and transverse microtubules: postciliary microtubules originate from the right and transverse microtubules arise from the left of each kinetosome (fig. 12).

The paroral membrane is a longtitudinal series of short (1-6 kinetosomes) oblique rows that lies within a pellicular groove on the right buccal overture (figs. 4-6). The rows are longer in the middle and shorter toward the anterior and posterior ends of the membrane. Postciliary microtubules originate near the right (posterior) kinetosome and transverse microtubules arise near the left (anterior) kinetosome of each oblique row (fig. 13). Nematodesmal microtubules plunge into the cytostome from the proximal part of the paroral kinetosomes; these fibers join with the postmembranellar fiber as it curves around the proximal membranelles and extends anteriorly (fig. 14).

Cirri. The midventral ciliature consist of 2 rows of parallelogram shaped cirri running along the longitudinal axis of the cell; each right midventral cirrus comprises 3 rows of 6-7 kinetosomes oriented at a 60° angle to the cell's longitudinal axis, whereas each left midventral cirrus is an assemblage of three rows of 5-6 kinetosomes lying at a 75° angle (figs. 16-19). These angles become less

Fig. 16, 17, 18, 19. Transmission electron micrographs of right midventral cirri (anterior is toward the top of the page in fig. 16, 17, toward the left side of the page in fig. 18, 19). Each anterior microtubular bundle (Amb) extends obliquely to a left midventral cirrus; right (Rmb) and left (Lmb) microtubular bundles extend toward the cell surface; the posterior microtubules (Pmb), along with postciliary microtubules (Pmt, fig. 19), course posteriorly below the cortex. Microtubular bundles originate from peripheral fibrillar material (Fm). Microtubular ribbing (Mtr) borders each cirrus and extends toward the cell surface. Single postciliary microtubules (arrows, fig. 19) are associated with triplet number 9 of internal cirral kinetosomes. (X 20 000, 20 200, 46 000, 46 000).


Rmb
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Amb
Mtr
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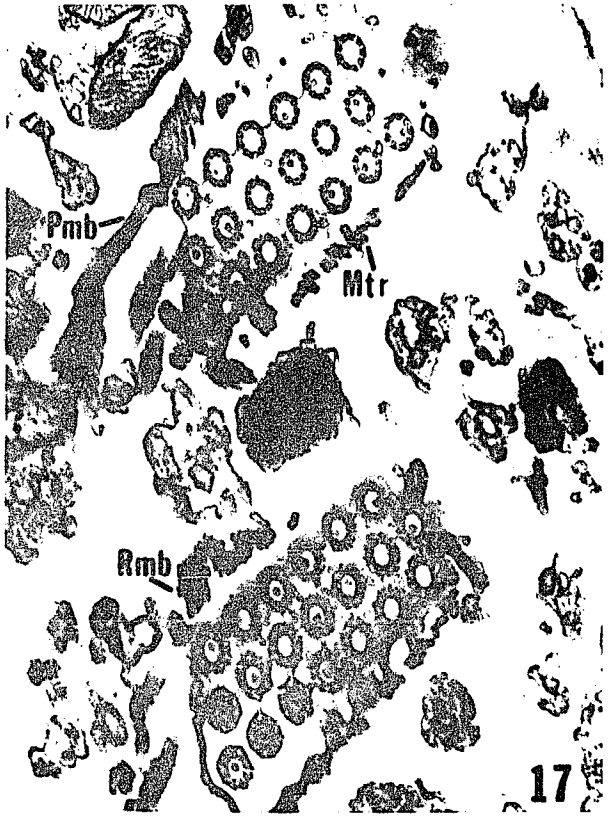
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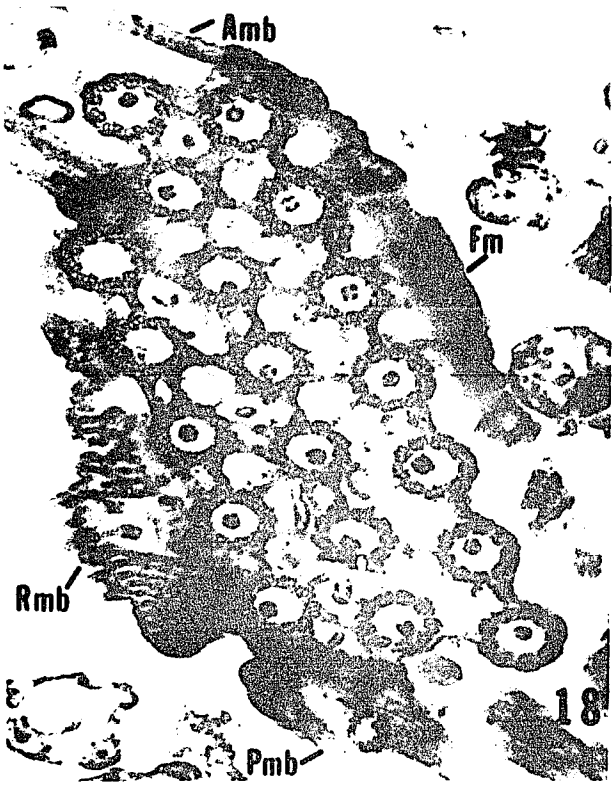
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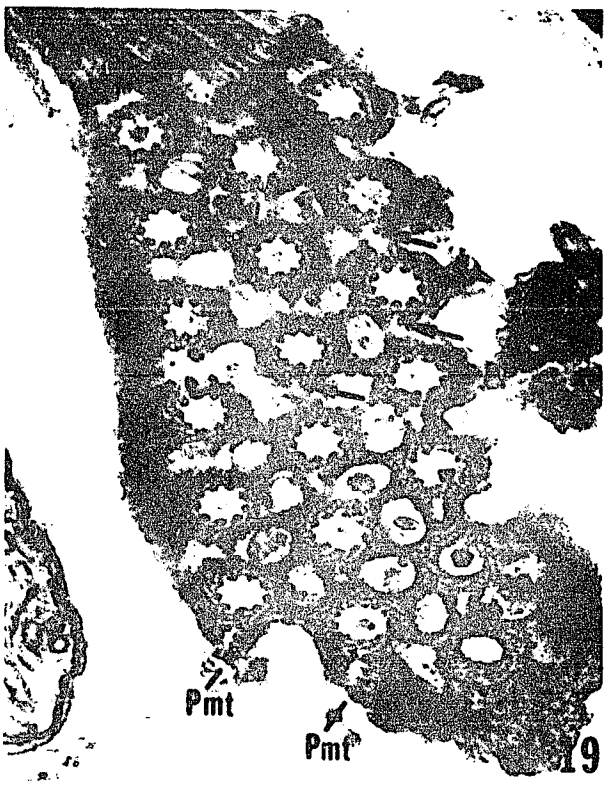

Pmt
Pmt
19

acute in cirri anterior to the cytostome. Thigmotactic cirral bases usually consist of 2 rows of 2-6 parallelogram arranged kinetosomes (although packets of 3 kinetosomes can also be found). Marginal cirri comprise 2 rows of 5-7 kinetosomes arranged at a 60° angle to the cell's main axis.

A closer look at a right midventral cirrus reveals each kinetosomal packet to be surrounded by an electron opaque microfibrillar matrix (figs. 16, 19). A similar microfibrillar system connects neighboring kinetosomes (figs. 16, 19). Four microtubular bundles originate from the microfibrillar peripheral matrix of the cirral base: an anterior bundle (7.5 μm), a posterior bundle (~6 μm), a right bundle (~5 μm), and a left bundle (~3 μm) (figs. 16-19). Posterior, right, and left microtubular bundles extend toward the cell surface while the anterior microtubular bundle runs parallel to the main axis of the cell, to join with the right posterior margin of the left midventral cirrus. In this way, right and left midventral cirri are linked in an oblique, ladder-like array.

Linear groups of microtubules, originating from the microfibrillar cirral matrix, border both anterior and posterior kinetosomal rows; these extend directly to the pellicle forming a basket-like framework around the cirrus base (figs. 16, 17). A similar system of microtubular ribbing borders thigmotactic and marginal cirri. Transverse microtubules arise from the far left kinetosomes of each cirrus and also extend directly toward the cell cortex. Postciliary microtubules originate at the far right kinetosomes, then join the posterior microtubular bundle as it courses at an oblique angle toward the cell surface (fig. 19). Single postciliary microtubules are associated with

triplet number 9 of internal cirral kinetosomes (fig. 19).

Morphogenesis

Cortical morphogenesis in Thigmokeronopsis occurs in 2 latitudinal developmental zones: an anterior field of the future proter and a posterior field of the future opisthe (figs. 20-31). The first morphogenetic event within these zones is initiation of oral primordia (OP) by proliferation of kinetosomes from the left midventral cirri in the opisthe and from the dedifferentiated endoral membrane in the proter (figs. 20, 23, 24, 31 b). Development occurs on the cell surface.

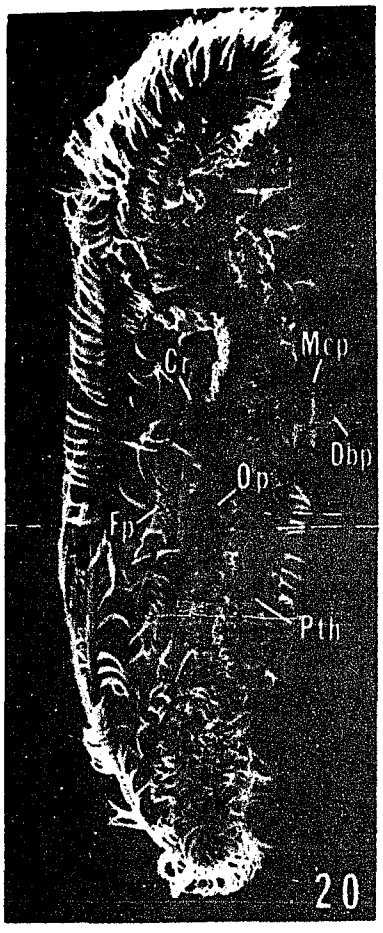
An undulating membrane primordium (UMP) forms at the right edge of the OP in both the proter and opisthe (figs. 20, 23, 24). At this stage membranelles begin to differentiate within the OP in a posteriad direction (membranellar organization in the opisthe proceeds that of the proter) (fig. 31). Paroral and endoral membranes differentiate from the UMP (fig. 29) and a paroral cirrus develops from the anterior end of the paroral membrane (fig. 31 f). Meanwhile, the membranelles and the endoral membrane form within, and the paroral membrane along the right side of, the new buccal cavity (figs. 22, 26-29). Both membranes are oriented with cilia directed outwardly (fig. 30). As development of the buccal cavity proceeds, the endoral membrane (probably because of cortical flow) becomes twisted, resulting in the cilia being directed inwardly toward the cytosome.

To the right of the proter OP, following the dedifferentiation of the paroral membarne and malar cirri, a frontal cirral primordium (FP) appears (figs. 20, 23, 31 c,d). As the FP develops, it elongates into a series of oblique streaks that lie within a newly formed cortical invagination (fig. 22).

Fig. 20, 21, 22. Scannning electron micrographs of early stages of cell division morphogenesis. In the opisthe, the oral primordium (Op) and the frontal primordium (Fp) are separated by a cortical ridge (Cr, fig. 21); both develop between midventral cirri and the parental thigmotactic cirri (Pth). Marginal cirral primordia (Mcp) and dorsal bristle primordia (Dbp) appear early in development (fig. 20, 21). The proter frontal primordium differentiates within a developing cortical invagination (Ci) while a buccal cavity (Bc) is formed in the opisthe (fig. 22). (X 650, 670, 600).

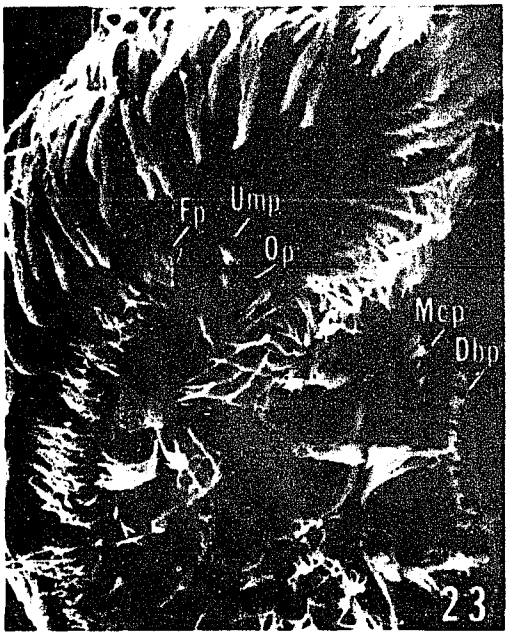
Fig. 23, 24. Details of figure 20. Both in the proter (fig. 23) and the opisthe (fig. 24), frontal (Fp), oral (Op), and undulating membrane (Ump) primordia are present. Marginal cirral primordia (Mcp) and dorsal bristle primordia (Dbp) appear closely associated. (X 1 600, 1 260).

Fig. 25. A portion of the dorsal suface of the same cell pictured in fig. 22 indicating the position of the opisthe's right marginal cirral primordium (Mcp) and right most dorsal bristle primordium (Dbp). (X 1 000).


Cr
Mcp
Dbp
Op
Fp
Pth
20


Fp
Mcp
Mcp
Dbp
21


Pth
Cr
Bc
22


Fp
Ump
Op
Mcp
Dbp
23


Op
Fp
Ump
24


Dbp
Mcp
25

Fig. 26, 27, 28. Scanning electron micrographs of late stages of cell division morphogenesis. The adoral zone of membranelles (Azm) and frontal streaks (Fs, fig. 27) have differentiated in both proter and opisthe; opisthe marginal cirri (Omc, fig. 26) and thigmotactic cirri (Oth, fig. 28) are differentiated. Parental membranelles (Pazm) and thigmotactic cirri (Pth) are resorbed during late development (fig. 28). (X 700, 700, 700).

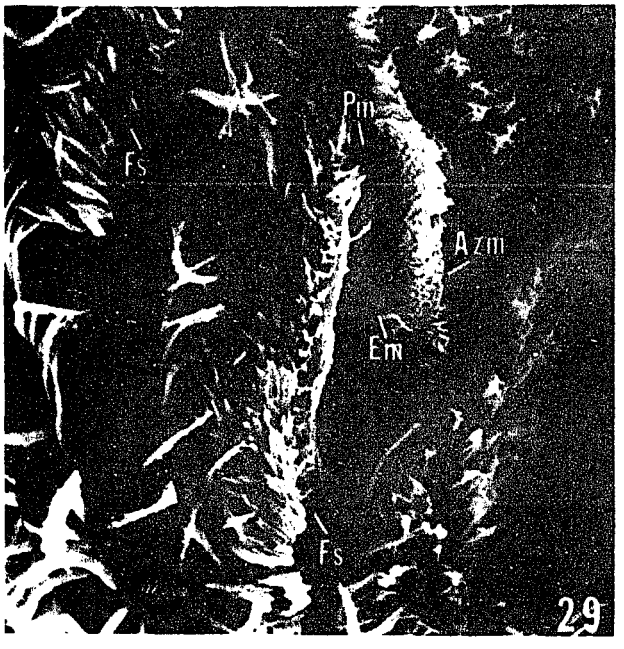
Fig. 29. A detail of the opisthe in fig. 27 showing the differentiated adoral membranelles (Azm), endoral (Em), and paroral (Pm) membranelles and frontal streaks (Fs). (X 1 300).

Fig. 30. A higher magnification of fig. 28 showing differentiated frontal ciliature in the opisthe: Mg - migratory cirri, Mv - midventral cirri, Tv - transverse cirri. (X 4 000).


Azm
Omc
Pth
26


Azm
Pazm
Fs
Azm
27


Pazm
Fs
Oth
Pth
28


Pm
Fs
Azm
Em
Fs
29

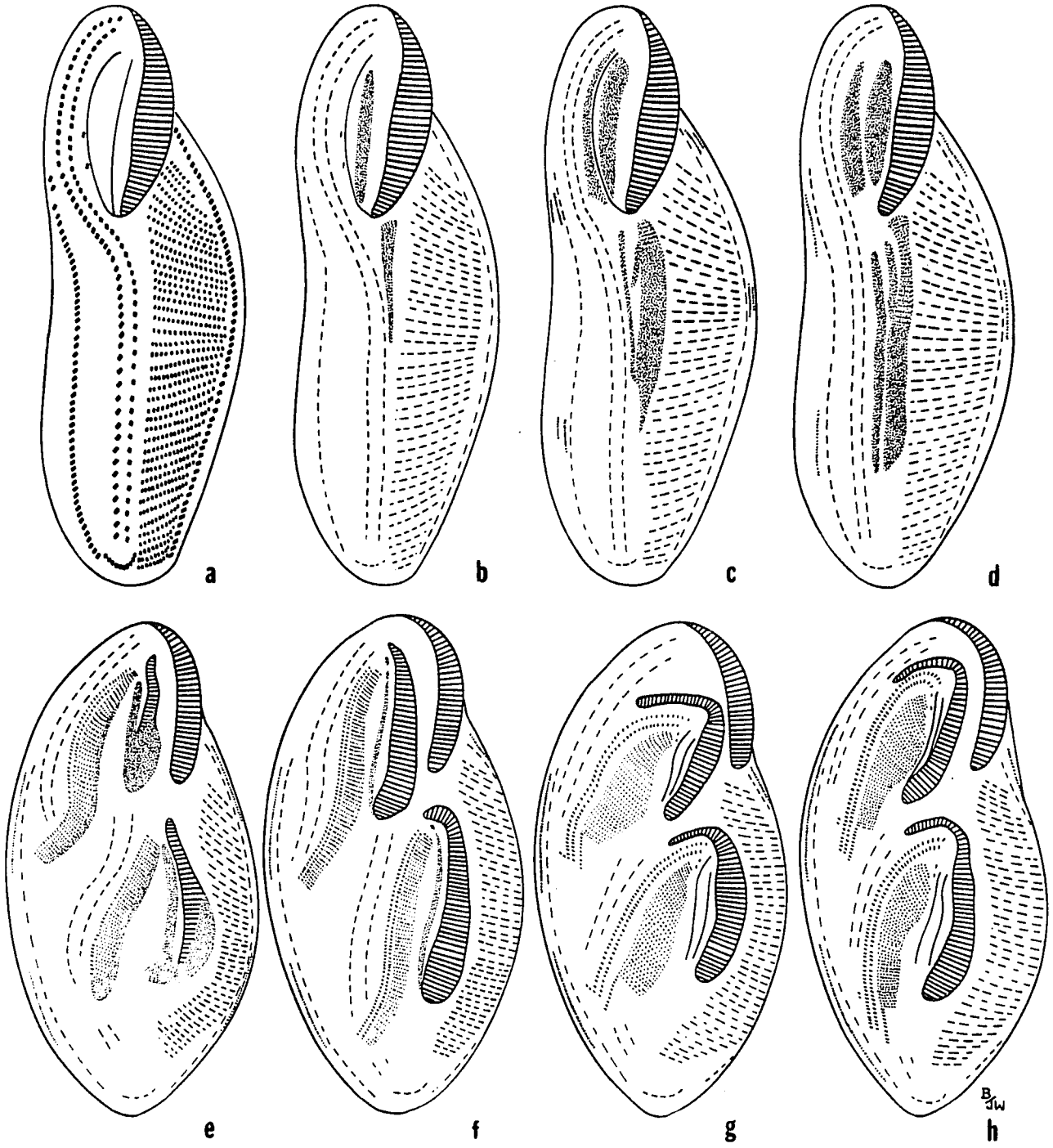

Mv
Mg
Tv

Two malar cirri differentiate from the anteriormost frontal streak; these eventually lie beside the paroral membrane. Three kinds of cirri differentiate from the last 10 frontal streaks: midventral, transverse and, from the last streak only, 2 additional cirri. These 2 migratory cirri are formed at the right edge of the last streak, then migrate anteriorly alongside the right midventral row until they are positioned behind the distal membranelles (figs. 29, 31 g,h). The 10 transverse cirri eventually subtend the midventral rows (fig. 31 e-h). In addition to midventral cirri, all remaining frontal streaks differentiate thigmotactic cirri (figs. 28, 31).

A similar FP develops alongside the midventral cirri in the opisthe. This field becomes separated from the OP by a cortical ridge, later to become the right buccal overture (fig. 20). Development of this FP proceeds as in the proter.

Marginal primordia (MP) and dorsal bristle primordia (DBP) appear early in the developmental process (figs. 20, 31 c). Both originate dorsal to old marginal cirral rows; right and left MP are closely associated with the first and last DBP (fig. 26).

Only after new sets of ciliature for both proter and opisthe have differentiated do the remaining parental structures begin to be disassembled and resorbed (fig. 28).



31

Fig. 31. Line diagrams of a non-dividing cell (fig. 31a) and a sequence of ventral, cortical morphogenetic stages during cell division (fig. 31b-h). Black lines and spots represent ciliary organelles.

Discussion

Ultrastructure

Cirri. A cirrus is an organellar complex of varying numbers of ciliferous kinetosomes embedded in a microfibrillar matrix; neighboring kinetosomes are linked (at 1 or 2 levels) by microfibrillar connections. Microtubular ribbons and bundles are associated with the external cirral kinetosomes or fibrillar matrix. Nemadesmata usually extend from the proximal end of cirral kinetosomes. A cirrus, therefore, represents an oligomerization by amplification of the kinetosomal components of an organellar complex (presumably with the decrease in total number of these complexes per cell), accompanied by the reduction of other components within the complex, such as microtubular ribbons.

The general structure of a cirrus is remarkably similar in all hypotrichs. Postciliary microtubular ribbons originate near the external right cirral kinetosomes in Oxytricha (GRIMES, 1972), Paraurostyla (GRIMES and L'HERNAULT, 1978), Gastrostyla (GRIM, 1972), Stylonychia (PUYTORAC et. al., 1976), Onychondromus (TUFFRAU et. al., 1968), and Discoephalus (WICKLOW, 1980) and in the heterotrich, Plagiotoma (ALBARET and GRAIN, 1973). A remnant postciliary microtubule (an oligomerized postciliary ribbon) is present at triplet number 9 of the internal cirral kinetosomes of some genera. Transverse microtubular ribbons arise from the exterior left kinetosomes in hypotrichs. These ribbons, although absent in cirri of Euplotes (TUFFRAU et. al., 1968), are present in the dorsal bristle complex of that genus (RUFFOLO, 1976b).

Kinetodesmal fibers also occur in hypotrich cirri; these fibers

are not always associated with every cirrus. As in Thigmokeronopsis, GRIMES has reported KD fibers only in some marginal and frontal cirri in Oxytricha (GRIMES, 1972). Later, GRIMES and ADLER (1976) observed a KD fiber only in the developing dorsal bristle complex. This finding is also consistent with the hypothesis of oligomerization as a phenomenon in the evolution of more complex ciliate structures.

A fourth kind of microtubular derivative occurs in Thigmokeronopsis: microtubular ribbing. These microtubular arrays that border anterior and posterior kinetosomal rows have not been observed in other hypotrichs. In Plagiotoma (ALBARET and GRAIN, 1968), however, the anterior and posterior cirral kinetosomes are bordered by similar linear microtubular arrays. In both ciliates the microtubules extend directly toward the cell surface; the dense fibrillar band at which the anterior microtubular array ends in Plagiotoma, however, is absent in Thigmokeronopsis.

Microtubular bundles (MTB) originating in the microfibrillar matrix around each cirrus are present in all hypotrichs. The point of origin of these bundles on the cirral base and their direction in the cytoplasm are different both for different kinds of cirri within a ciliate species and between cirri of different species. MTBs of marginal cirri, although uniform within the row, differe significantly from frontal cirri of the same cell. In Stylonychia (TUFFRAU, 1965), MTBs of right marginal cirri differ both in number and orientation from frontal cirral MTBs. Between different ciliate species marginal cirral MTBs appear very conservative. For example, in Stylonychia (TUFFRAU, 1965), Paraurostyla (GRIMES and L'HERNAULT, 1978), and Gastrostyla (GRIM, 1972) right marginal cirral MTBs are so similar as to be homologous.

Frontal cirral MTB organization, although varying in different regions of the same cell, may reveal homologies between cells. For instance, anterior frontal cirri in Stylonychia (TUFFRAU, 1965), Paraurostyla (GRIMES and L'HERNAULT, 1978), and Oxytricha (GRIMES, 1972) all possess a posterior MTB and radiating anterior MTBs that attach to the collar membranelles. This homology may reflect a close systematic relationship between these genera (BORROR, 1972, 1979b).

However, in Thigmokeronopsis the midventral cirral MTB organization is uniform throughout the cell in contrast to the "sporadically" positioned frontal ciliature of the 3 species mentioned above. Attachment of the left midventral, anterior MTB to the right midventral cirral base contributes to this uniformity. Midventral cirral rows are a structurally, functionally, and developmentally cohesive unit and a distinct evolutionary exploitation of frontal ciliature.

Paroral apparatus. Ultrastructure of ciliate oral organelles was reviewed by PUYTORAC and GRAIN in 1975 and PUYTORAC et. al. in 1976; I use their terminology in the following.

A double paroral appraatus exists in all hypotrichs and some heterotrichs: the endoral membrane always consists of a row of single kinetosomes (stichomonade); the paroral membrane is variable. The paroral membrane ranges from a row of single kinetosomes (together with the endoral row termed diplostichomonade) found in Oxytricha (GRIMES, 1972), Stylonchia (PUYTORAC et. al., 1976), Caenomorpha (RODRIGUES DE SANTA ROSA, 1976), Nyctotherus (PAULIN, 1967), and Plagiotoma (ALBARET and GRAIN, 1973) to a row of multiple arrays of 2-6 kinetosomes (polystichomonade) as is present in Gastrostyl (GRIM, 1972), Swedmarkia (LUPORINI and MAGAGNINI, 1970), Paraurostyla (BAKOWSKA and

JERKA-DZIADOSZ, 1978), and Thigmokeronopsis. The number of kinetosomes within each array of the paroral row varies both within and between ciliate families.

Oligotrichs, Halteria (GRAIN, 1972) and Petalotricha (LAVAL, 1972), possess a single membrane paroral apparatus (stichomonade). The paroal apparatus of the heterotrichs, Climacostomum (PECK et. al., 1975) and Stentor (PUYTORAC et. al., 1976) consists of a row of kinetosome pairs reminiscent of oral structures in some pleurostomes.

Adoral apparatus. Hypotrich ventral (lapel) paramembranelles each comprise 4 rows of kinetosomes oriented at a 90° angle to the longitudinal axis of the membranellar zone; the first (posteriormost) and second rows of each paramembranelle are of equal length and are the longest rows, the third is slightly shorter, and the fourth (anteriormost) is shortest, consisting of 6 (or less) kinetosomes located at the extreme right of each paramembranelle. Frontal (collar) paramembranelles have been described at the ultrastructural level only in Paraurostyla (BAKOWSKA and JERKA-DZIADOSZ, 1978). Each also consists of 4 rows of kinetosomes: row 1 and 2 are shorter than in ventral paramembranelles (more similar in size to row 3), and the short, fourth row is displaced toward the middle of the membranelle.

Transverse microtubular ribbons originate from kinetosomes of the fourth membranellar row. In Stylonychia (PUYTORAC et. al, 1976), as in Thigmokeronopsis, additional transverse microtubular ribbons are associated with the third membranellar row; electron micrographs of Oxytricha (GRIMES, 1972) and Gastrostyla (GRIM, 1972) reveal a remnant transverse microtubule associated with each kinetosome in this row. Transverse microtubules have not been observed in the

third membranellar row in Paraurostyla (BAKOWSKA and JERKA-DZIADOSZ, 1978).

Postciliary microtubular ribbons are always, but not only, associated with kinetosomes of the first membranellar row. Single postciliary microtubules are present in Paraurostyla (BAKOWSKA and JERKA-DZIADOSZ, 1978) and are evident, although not referred to, in electron micrographs of Oxytricha (GRIMES, 1972), Stylonychia (PUYTORAC et. al., 1976), Gastrostyla (GRIM, 1972), and the heterotrich Plagiotoma (ALBARET and GRAIN, 1973), in association with triplet number 9 of each kinetosome of membranellar rows 2, 3, and 4. These remnant (oligomerized) microtubular ribbons may be clues to the origin of the polyhymenophora (see below).

In the heterotrichines Climacostomum (PECK et. al., 1975) and Condylostoma (BOHATIER, 1978), and in the plagiotomine Plagiotoma (ALBARET and GRAIN, 1973), paramembranelles comprise just 3 rows of kinetosomes; transverse microtubular ribbons arise from row 3 in Climacostomum and Plagiotoma, but are completely absent in paramembranelles of Condylostoma. There are 4 rows of kinetosomes in the paramembranelles of the armorphorine Caenomorpha (RODRIGUES DE SANTA ROSA, 1976): transverse microtubules arise both from the kinetosomes of row 1 and from the far right kinetosomes of each membranellar row.

The cirromembranelles of the colpopids Woodruffia (GOLDER and LYNN, 1980), Kuklikophrya (NJINE, 1979), and Platyophora (DRAGESCO et. al., 1977) consist of 2 kinetosomal rows, while those in Bryophrya (GRAIN et. al., 1979) and Bursaria (PEREZ-PANIAGUA et. al., 1980) consist of 3 kinetosomal rows. In Bryophrya and Bursaria, each

kinetosome of the membranelle possesses a postciliary microtubular ribbon; in Bryophrya, some of the internal kinetosomes of the membranelle retain only a single, remnant postciliary microtubule. Interkinetosomal fibrils and nematodesmal microtubules are also present. Transverse microtubules are totally absent.

PEREZ-PANIAGUA et. al. (1980) concluded the cirromembranelles of the colpopids to be non-homologous with the paramembranelles of the polyhymenophora on the basis of the number of membranellar rows possessing postciliary microtubular ribbons (supposedly only one in paramembranelles and all rows in cirromembranelles) and by the complete absence of transverse microtubules in cirromembranelles. Both types of membranelles have significantly diverged from one another as predicted by the structural conservatism hypothesis (LYNN, 1976). There exist, however, similarities between paramembranelles and cirromembranelles that may prove to be homologous. For example, paramembranelles share with cirromembranelles the presence of, at least reduced, postciliary ribbons in all kinetosomal rows; transverse microtubules are associated with most paramembranelles but are completely lacking (as in cirromembranelles) in the paramembranelles of Condylostoma. Although more information is necessary (an ultrastructural analysis of cirromembranellar stomatogenesis would be most helpful), the above similarities may reflect an unsuspected common ancestry. Perhaps the polyhymenophora diverged from a colpodid lineage.

Morphogenesis

Three kinds of primordia appear during cell division morphogenesis in all hypotrichs: within-row or somatic, frontal and oral.

Additionally a fourth type, a ventral primordium (described below), occurs in only Pseudourostyla and Epiclintes. Development of somatic kineties occurs within dorsal bristle rows and marginal cirral rows. GRIMES and L'HERNAULT (1978) have shown that ventral longitudinal cirral rows in Paraurostyla hymenophora arise by within-row development, hence are homologous to marginal cirral rows (somatic ciliature) and are different from midventral cirral rows (frontal ciliature). Oral primordia may arise de novo or by dedifferentiation of pre-existing structures. Frontal primordia are frequently associated with developing oral primordia but can arise independently by dedifferentiation of parental structures. Within-row development, present in many ciliate classes, represents an ancestral characteristic or plesioseme; frontal primordial development, unique to the polyhymenophera, represents a divergent trait or aposeme. Plesioseme and aposeme are terms coined by HANSON (1976).

Some aspects of heterotrich ciliature are homologous to that of hypotrichs. In Climacostomum (DUBOCHET et. al., 1979) an oral primordium develops by kinetosome proliferation of postoral kineties; this divides into a membranellar anlage on the left and a paroral anlage on the right. Three types of ciliature differentiate from the paroral field: peristomial field kineties on the left, apical membranelles anteriorly, and a "haplokinety" on the right.

In addition to the homology between paramembranelles of heterotrichs and hypotrichs, peristomial ciliature in Climacostomum is homologous to hypotrich frontal ciliature; apical membranelles, also found in Condylostoma (BOHATIER et. al., 1976), are homologous to hypotrich paroral cirri. A homology may also exist between the

haplokinety in Climacostum, the undulating membrane of Condylostoma and the endoral membrane of hypotrichs.

Although sharing homologous somatic, frontal and oral structures, hypotrichs display several distinct developmental patterns during the formation of these organelles. Examples of divergent developmental patterns include those reported in Discocephalus (WICKLOW, 1978), Epiclintes (WICKLOW, 1979), Euplotes (RUFFOLO, 1976a), Kahliella (TUFFRAU, 1969), Oxytricha (GRIMES, 1972), and Urostyla (BORROR, 1979b).

BORROR (1979b) demonstrated marked differences in both structure and morphogenetic patterns in genera that were formerly considered Urostylids. This indicated a polyphyletic, hence artificial, grouping. Borror, in redefining this family, concluded that only those genera possessing midventral cirri developing from an oblique series of frontal streaks should be considered Urostylids: Bakuella, Holosticha, Keronopsis, Pseudourostyla, Uroleptus, and Urostyla. Thigmokeronopsis jahodai represents an additional member of this group.

Cortical morphogenesis during cell division in Thigmokeronopsis is summarized in figure 31. The development of the proter oral primordium is associated with the differentiation of the endoral membrane; the development of the proter frontal primordium is associated with the differentiation of the paroral membrane and malar cirri. As in Urostyla (JERKA-DZIADOSZ, 1972), Holosticha (under the name Keronopsis in JERKA-DZIADOSZ and JANUS, 1972), Pseudourostyla JERKA-DZIADOSZ, 1972), and Keronopsis (WICKLOW, unpublished), the opisthe oral primordium in Thigmokeronopsis develops by proliferation of kinetosomes from left midventral cirri. The opisthe frontal pri-

mordia also arises from kinetosomes of midventral cirri. Like other Urostylids, frontal primordia develop into a longitudinal series of oblique streaks. Migratory cirri, first observed during division morphogenesis in H. scutellum (HILL, 1979), differentiate from the posteriormost frontal streak. Midventral cirri and transverse cirri also differentiate from this streak. Keronopsis also possesses migratory cirri that develop from the posteriormost frontal streak; also present is a series of about 6 accessory transverse cirri of the last 10 frontal streaks (WICKLOW, unpublished).

Anterior frontal streaks differentiate hypertrophied midventral cirri in Bakuella, Holosticha, Uroleptus, and Urostyla; in Thigmokeronopsis, Keronopsis, and Pseudourostyla all midventral cirri are of equal size. Thigmokeronopsis is the only Urostyline possessing a thigmotactic cirral field.

Marginal cirral primordia arise by within-row development in Thigmokeronopsis; this phenomenon is shared by all Urostylids except Pseudourostyla. The 14 ventral longitudinal rows in Pseudourostyla arise by a right and left primordium in both proter and opisthe (JERKA-DZIADOSZ and JANUS, 1972). Each primordium forms a series of 7 oblique streaks that differentiate into longitudinal rows. Epiclintes, although lacking midventral cirri, possesses similar ventral primordia that give rise to all its ventral longtitudinal rows of cirri (WICKLOW, 1979). The longitudinal rows in both Pseudourostyla and Epiclintes are clearly not homologous with marginal cirral rows of other hypotrichs. Ventral primordia represent a fourth type of developmental field occuring during cell division morphogenesis in hypotrichs.

Phylogenetic Implications

Cirri and paramembranelles. Cirri and paramembranelles are oligomerized organellar complexes; this condition is presumed to be the result of a polymerization of homologous organelles followed by fusion, reduction or change in function of homologous structures during the evolutionary history of the organism (see POLJANSKI and RAIKOV, 1976).

The morphogenesis of an organellar complex may reveal clues to its evolutionary history. During stomatogenesis in Oxytricha (GRIMES, 1972), the immature paramembranelles consist of a transverse row of kinetosomal pairs; after this initial kinetosomal alignment (occuring from left to right, beginning anteriorly and proceeding posteriorly within the OP) new kinetosomes are added, from left to right, at the anterior margin of each pair. The fourth membranellar row is presumed to be added in the same fashion. According to Grimes, a similar sequence of events occurs during cirral development. In both instances, the interkinetosomal fibrillar connections are added during the later stages of development.

Cirri and paramembranelles represent kinetosomal pairs that polymerize by addition of kinetosomes, thus representing oligomerized homologues of somatic kineties found in other ciliates. Kinetosomal pairs comprise the somatic kineties of some heterotrichs, colpodids and karyorelectids as well as the dorsal bristle complex in hypotrichs.

A progressive shift of the cytostome from an apical to a ventral position has occured during ciliate evolution. This transition was accompanied by a concomitant shift of the pre-oral somatic kineties; these kineties eventually lie at a right angle to their original

longitudinal axis. This phenomenon accounts for the transverse position of the paramembranelles. Each paramembranelle is a kinety (albeit modified) complete with postciliary and transverse microtubular ribbons; its anterior end is now positioned along the left border of the buccal cavity.

Postciliodesmata are present in the somatic kineties of members of the Karyorelictida and the Heterotrichina (GIERASSIMOVA and SERAVIN, 1976). The overlapping postciliary ribbons from each paramembranelle that form the postmembranellar fiber in hypotrichs can be interpreted as a modified postciliodesma.

Urostyline phylogeny. Two different developmental patterns occur during cell division morphogenesis in Urostylines that indicate phylogenetic divergence (fig. 32). In one group, 3 kinds of primordia are present: oral, frontal, and somatic (within row); all non-midventral, longitudinal cirral rows arise by within row development, hence are considered marginal cirral rows. This group includes Holosticha, Bakuella, Keronopsis, Thigmokeronopsis, Uroleptus, and Urostyla.

Within this group a second divergence has occurred (fig. 32). In one subgroup, represented by Holosticha, Bakuella, Uroleptus, and Urostyla, the anterior frontal cirri have differentiated from other midventral cirri becoming markedly hypertrophied. The distal end of the adoral zone of paramembranelles in these genera extends only to the anterior end of the cell. In the second subgroup, represented by Keronopsis and Thigmokeronopsis, no differentiation of midventral cirri has occurred - all midventral cirri are of equal size. The distal paramembranelles of these genera extend past the anterior end of the cell to a point along the right lateral border of the cell.

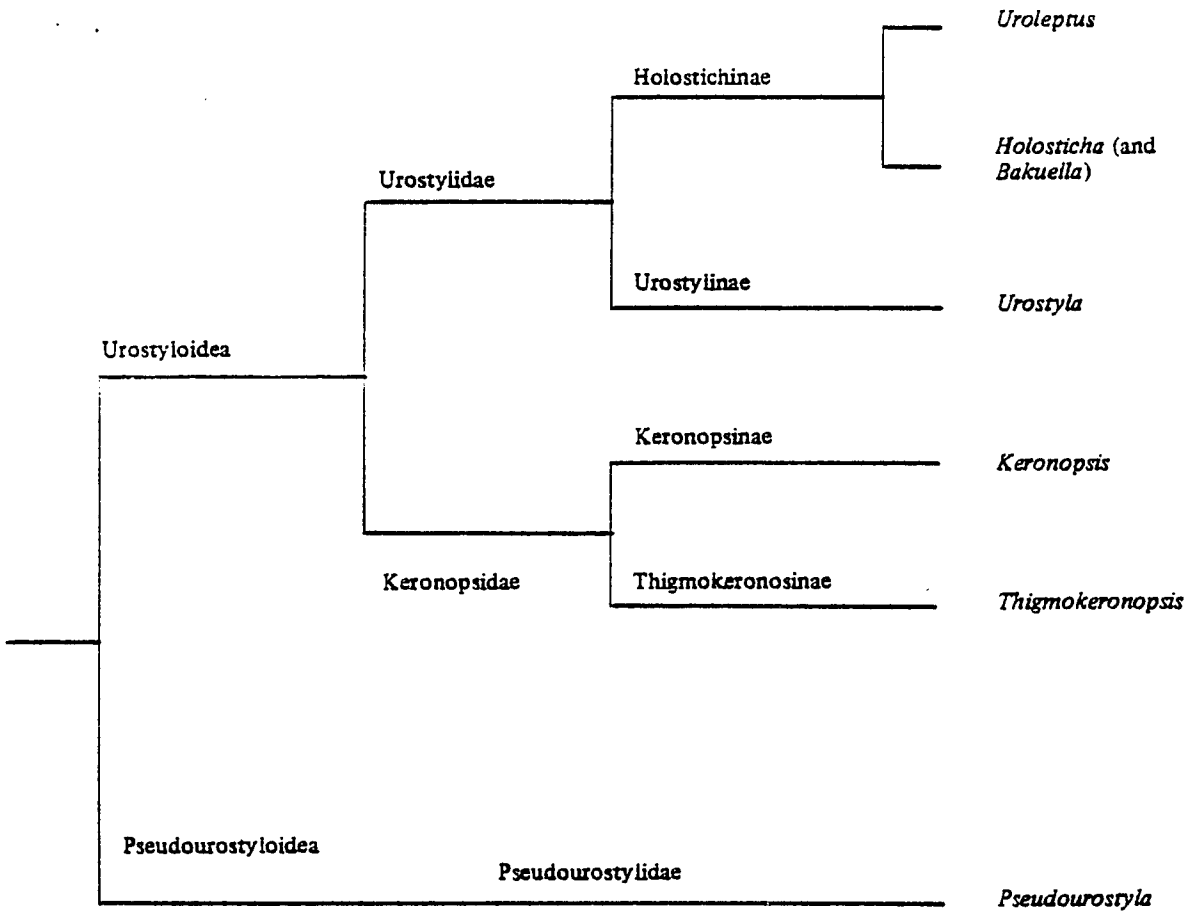


Fig. 32. A proposed phylogeny of Urostyline hypotrichs based on structural and morphogenetic data.

The second developmental pattern occurs in a group that possesses, in addition to oral, frontal and somatic primordia, a fourth type - ventral primordia. All non-midventral, longitudinal cirral rows arise from the ventral primordia: no true marginal cirral rows are present. The differences between these two groups indicates a phylogenetic gap that I believe warrants separation at the superfamilial level.

Epiclintes also possesses ventral primordia (WICKLOW, 1979). Although its frontal ciliature is markedly reduced (midventral cirri are completely lacking) it may have diverged from a Pseudourostyla-like ancestor. More ultrastructural data is needed to clarify the systematic position of Epiclintes.

JANKOWSKI, in his 1979 revision of the Hypotrichida, suggests 6 new suborders: Urostylina, Holostichina, Oxytrichina, Euplotina, Gastrocirrihina, and Apidiscina. I shall limit my discussion to the suborders Urostylina and Holostichina.

The Urostylina, according to JANKOWSKI, includes the Urostylidae, Keronopsidae, Psilotrichidae, Kiitrichidae, Strongyliidae, Atractidae, Spirofilopsidae, Hypotrichidiidae, Spiretellidae, and Chaetospiridae. Both the Urostylids and Keronopsids possess midventral ciliature; all of the remaining families within this proposed group do not, thereby rendering the group polyphyletic. In order to maintain a monophylectic classification, these remaining families cannot be assigned to the Urostylina. Their systematic position is beyond the scope of this paper.

JANKOWSKI also proposed the suborder Holostichina, including the families: Holostichidae, Bakuellidae, Pseudourostylidae, and

Banyulsellidae. There is no morphogenetic information regarding the family Banyulsellidae (represented by a single genus and species), hence, its systematic position remains uncertain. Bakuella does not differ significantly in structure or morphogenesis from Holosticha, thus its separation at the familial level is unjustified. BORROR (1979) demonstrated the morphological and morphogenetic similarities between Holosticha and Urostyla; he therefore dropped the name Holostichidae as a junior synonym. I consider Holosticha and Bakuella as belonging to the subfamily Holostichinae in the family Urostylidae. The erection of the order Holostichina is therefore unwarranted. The morphogenetic pattern of Pseudourostyla differs significantly from that of other Urostylines. It should be separated into its own superfamily: the Pseudourostyloidea.

Diagnosis of the families and subfamilies of the Urostylina

Order Hypotrichida Stein, 1859

Suborder Urostylina Jankowski, 1979

Diagnosis. Frontal ciliature includes midventral cirri that develop during division morphogenesis from a longitudinal series of oblique streaks; somatic ciliature includes dorsal bristle rows and marginal cirral rows (marginal cirri are replaced in one group by longitudinal ventral cirral rows that differentiate from ventral primordia.

Superfamily Urostyloidea Butschli, 1889

Diagnosis. In addition to midventral cirri, 5 other frontal derivatives may be present: malar, migratory, transverse, accessory transverse, and thigmotactic cirri. All non-midventral, longitudinal

cirral rows arise by somatic (within row) development and are considered marginal cirral rows.

Family Urostylidae Butschli, 1889

Diagnosis. Anterior frontal cirri are differentiated from other midventral cirri becoming markedly hypertrophied; the distal end of the adoral zone of paramembranelles extends only to the anterior of the cell.

Subfamily Holostichinae (n. subfam.)

Diagnosis. In addition to midventral and one or more left marginal cirral rows, only one right marginal cirral row is present. Holosticha, Bakuella, Uroleptus.

Subfamily Urostylinae (n. subfam.)

Diagnosis. In addition to midventral and left marginal cirral rows, several right marginal cirral rows are present. Urostyla.

Family Keronopsidae Jankowski, 1979

Diagnosis. All midventral cirri are of equal size (no hypertrophy of anterior frontal cirri has occurred). Distal paramembranelles extend past the anterior end of the cell to a point along the right lateral cell border.

Subfamily Keronopsinae (n. subfam.)

Diagnosis. Frontal ciliature may comprise of malar, migratory, midventral and transverse cirri; a thigmotactic cirral field is absent. Keronopsis.

Subfamily Thigmokeronopsinae (n. subfam.)

Diagnosis. In addition to malar, migratory, midventral and transverse cirri, a thigmotactic cirral field is present. Thigmokeronopsis.

Thigmokeronopsis n. gen.

Diagnosis. Somatic ciliature includes dorsal bristle rows, one left and one right marginal cirral row; frontal ciliature includes migratory, midventral, transverse, malar, and thigmotactic cirri. Thigmotactic cirri form a left, post-oral ciliary field used for adhesion to substrate. Multimacronucleate.

Superfamily Pseudourostyloidea (n. superfam.)

Diagnosis. In addition to midventral cirri, malar and transverse cirri also differentiate from frontal streaks. Marginal cirri are absent; all non-midventral, longitudinal cirral rows develop from ventral primordia.

Family Pseudourostylidae Jankowski, 1979

Diagnosis. As above. Includes one genus, Pseudourostyla.

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THE DISCOCEPHALINA (N.SUBORD.): ULTRASTRUCTURE, MORPHOGENESIS AND EVOLUTIONARY IMPLICATIONS OF A GROUP OF ENDEMIC MARINE INTERSTITIAL HYPOTRICHS (CILIOPHORA, PROTOZOA).

CHAPTER II

THE DISCOCEPHALINA (N.SUBORD.): ULTRASTRUCTURE, MORPHOGENESIS, AND EVOLUTIONARY IMPLICATIONS OF A GROUP OF ENDEMIC MARINE INTERSTITIAL HYPOTRICHS (CILIOPHORA, PROTOZOA)

Introduction

The interstices of marine sands represent a dynamic yet stable habitat that harbors a diverse community of ciliates ranging from those considered among the most "primitive" to those considered among the most "advanced" of the phylum. Ciliates living within this conservative milieu show a number of convergent morphological, behavioral, and life history adaptations (BORROR, 1980; CORLISS and HARTWIG, 1977; DRAGESCO, 1962; FAURE-FREMIET, 1951; FENCHEL, 1978). Cells are predominantly narrow or flattened and elongate (Geleia, Remanella), circular or oval, (Aspidisca, Diophrys), and/or strongly cephalized (Discocephalus); fragility and contractility is common in many elongate species while high cytoskeletal complexity and cortical rigidity is shared by oval species. Ciliature is often limited to the ventral surfaces (e.g. Trachelonema) and, in some species, is greatly hypertrophied (e.g. Discotricha). Supposedly primitive diploid macronuclei occur in many species (karyorelictids) and polygenomic multimacronuclearity is common in others (hypotrichs); thigmotaxis and the absence of encystment is generally characteristic of endemic psammolittoral species. The biology, including morphogenesis and ultrastructure, of most of the 600 known (Dragesco, 1962) interstitial ciliates has yet to be studied.

Because of their constancy through time, marine sands shelter forms that may have persisted with little change for hundreds of millions of years and, hence, may hold clues to help unravel mysteries of ciliate evolution. CORLISS and HARTWIG (1977) employ this hypothesis in examining the position of the "primitive" karyorelictids in the evolution and systematics of the Ciliophora. Because hypotrichs are considered at the "peak" of ciliate evolution, their study can also enhance our understanding of evolutionary processes and trends within the phylum. For example, ultrastructural and morphogenetic studies have demonstrated hypotrich somatic cirri to be oligomerized homologues of the somatic ciliature of heterotrich and even karyorelictid ciliates. Furthermore, there is fossil evidence that tintinnine oligotrichs, at the spirotrich evolutionary grade, occurred at least 400 million years ago (TAPPAN and LOEBLICH, 1968). Marine, psammophilic hypotrichs, also at this advanced spirotrich grade, may therefore represent evolutionary lineages at least as ancient as the early Paleozoic.

The paucity of fossil evidence in nonloricate ciliates, however, renders such tools as ultrastructure and morphogenesis vital to the study of ciliates hypothesized as ancestral or evolutionary intermediate forms and their use in constructing natural ciliate phylogenies. LIPSCOMB and CORLISS (1978) and CORLISS (1979b) used these tools to advantage in the study of the unique and once phylogenetically puzzling Stephanopogon.

The present paper is a study of a group of ciliates endemic to marine interstitial environments, the Discocephalus-like hypotrichs. In it I describe ultrastructure and morphogenesis of Discocephalus

ehrenbergi and morphogenesis in Amphisiella marioni and Psammocephalus faurei n.comb.; I synoptically review previously described discocephalids as well as describe a new discocephalid genus with 2 new species. With these newly described species serving as evolutionary intermediates, I demonstrate an evolutionary series within the group, and suggest phylogenetic relationships between the discocephalids and other ciliate taxa. Finally, I propose a classification of Discocephalus-like hypotrichs with changes up to the subordinal level.

Materials and Methods

I isolated, for use in morphological (including ultrastructural) and morphogenetic studies, Discocephalus ehrenbergi Dragesco, 1960, Psammocephalus borrori n.gen., n.sp., and Psammocephalus dragescoi n.gen., n.sp. from intertidal sands of Foss Beach, New Hampshire. I collected samples from the first 6 cm of sand between mean tidal level and mean low water for both Uhlig extraction (UHLIG, 1965) and culture; subsamples of sand were maintained as mass cultures that I enriched with F_2 medium (GUILLARD and RYTHER, 1962) and kept at the original temperature (8°C) and salinity (32%). I isolated Psammocephalus faurei n.comb. from sands collected near mean low water at Plum Island, Massachusetts and maintained mass cultures as above, at 16°C (S=32%). I subcultured Amphisiella marioni from a sample collected on the coast of the Yucatan peninsula, Mexico; populations were grown on the diatom Phaeodactylum in 38% seawater at 16°C.

I observed additional populations of D. ehrenbergi, for use in studying morphological variation, from sand samples from Santa Barbara, California, St. Ann's Bay, Nova Scotia, and the seawater tables at Jackson Estuarine Laboratory on Great Bay, New Hampshire. I have also studied protargol stained specimens (courtesy of Dr. Jean Dragesco) from Roscoff and Arcachon, France.

I observed cells live as well as stained using a modification of Tuffrau's (1967) protargol technique. This modification and my procedure for processing cells for S.E.M. and T.E.M. are described

in WICKLOW (1981a).

All references to the cell will be relative to the cell's left or right; in ventral aspect the cell's left corresponds to the reader's right.

Type specimens for newly described species are kept as part of my permanent slide collection.

Results

Discocephalus ehrenbergi Dragesco, 1960.

Behavior and Ecology

D. ehrenbergi feeds on diatoms and algae (frequently ingesting small chips of sand) by traveling on and between sand grains in a slow forward, then erratic backward motion, flexing the cephalized region to conform to the irregular contours of each sand grain. This cell is highly thigmotactic: when disturbed it can either adhere firmly to the substrate or spiral rapidly backward, propulsed by its large transverse cirri, then settle suddenly and attach or begin feeding again. Generally this ciliate is associated with the upper 3 cm of sand.

General Morphology

D. ehrenbergi is stout, ranging from 40-100 μm in length, 23-50 μm in width (fig. 1). A morphometric light microscopic description based on protargol stained individuals from different populations is presented in Table 1. The cell is oval with a strongly cephalized anterior end; this cephalized region forms a ventral, lip-like, peristomal lobe and houses the oral apparatus as well as several dorsal and lateral spine-like protrusions (described below) (figs. 2-5). A midventral concavity and several cortical grooves are present (as observed in fixed and living cells); the dorsal surface is convex, tapering posteriorly, then ending with a short tail or rump (figs. 2, 3). Although the pellicle is rigid, the cephalized region can be flexed.

Table 1. Geographic variation in D. ehrenbergi DRAGESCO, 1960.

Character		Roscoff,* France	Arcachon,* France	Foss Beach, N.H.	J.E.L., N.H.	St. Ann's Bay, Nova Scotia	Santa Barbara, C.A.
n		4	6	20	20	20	2
somatic length	$\bar{x}$ µm	92.5	85	64.95	66.25	47.4	52.5
	range	85-100	75-100	55-75	50-85	40-60	50-55
somatic width	$\bar{x}$ µm	47.5	45.5	38.3	38.3	27.6	27
	range	45-50	43-50	27-50	30-50	23-35	
collar (dorsal) membranelles	$\bar{x}$	6	6	5.9	6	6	6.5
	range			5-6			6-7
lapel (ventral) membranelles	$\bar{x}$	18.25	19.6	18.4	20.1	18.95	13
	range	17-19	18-20	18-19	18-23	18-22	11-15
anterior left marginal cirri	$\bar{x}$	2	2	1.95	2	2	2
	range			1-2			
posterolateral left marginal cirri	$\bar{x}$	11.75	13.6	11.6	13.4	11.25	11
	range	11-12	12-14	7-14	11-15	10-13	10-12
transverse cirri	$\bar{x}$	7	8	8.05	8.95	8.05	8
	range			8-9	8-10	8-9	
caudal cirral sets	$\bar{x}$	3	3.3	4	3.15	4	4
	range		3-4		3-4		
dorsal kineties		6	6	6	6	6	6

Blank spaces indicate character is constant for the population.
*measurements based on material prepared by Dr. J. Dragesco

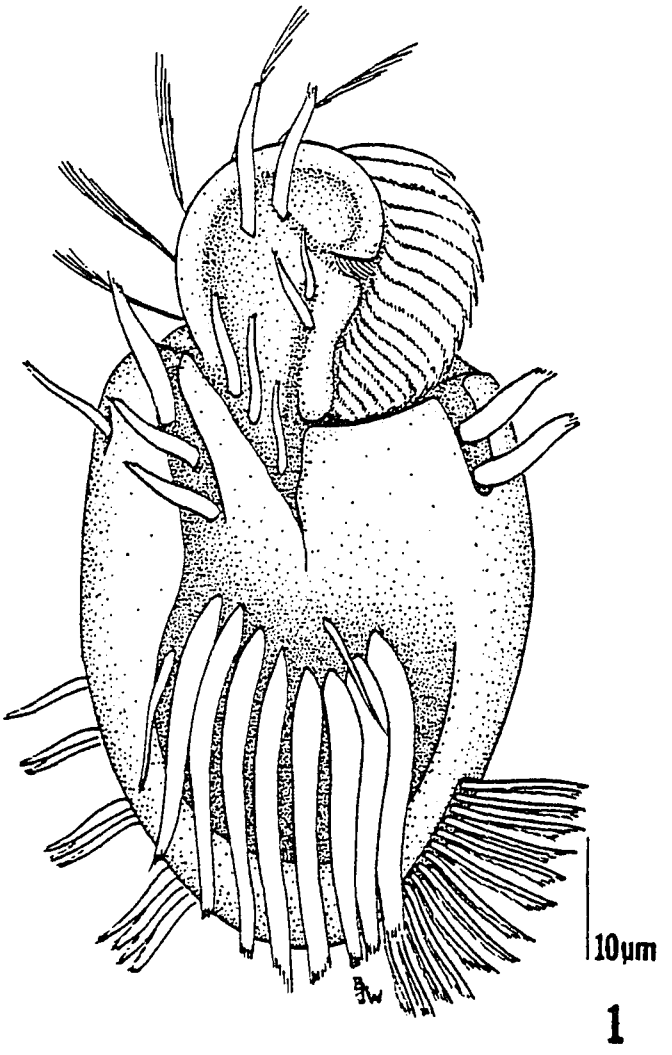
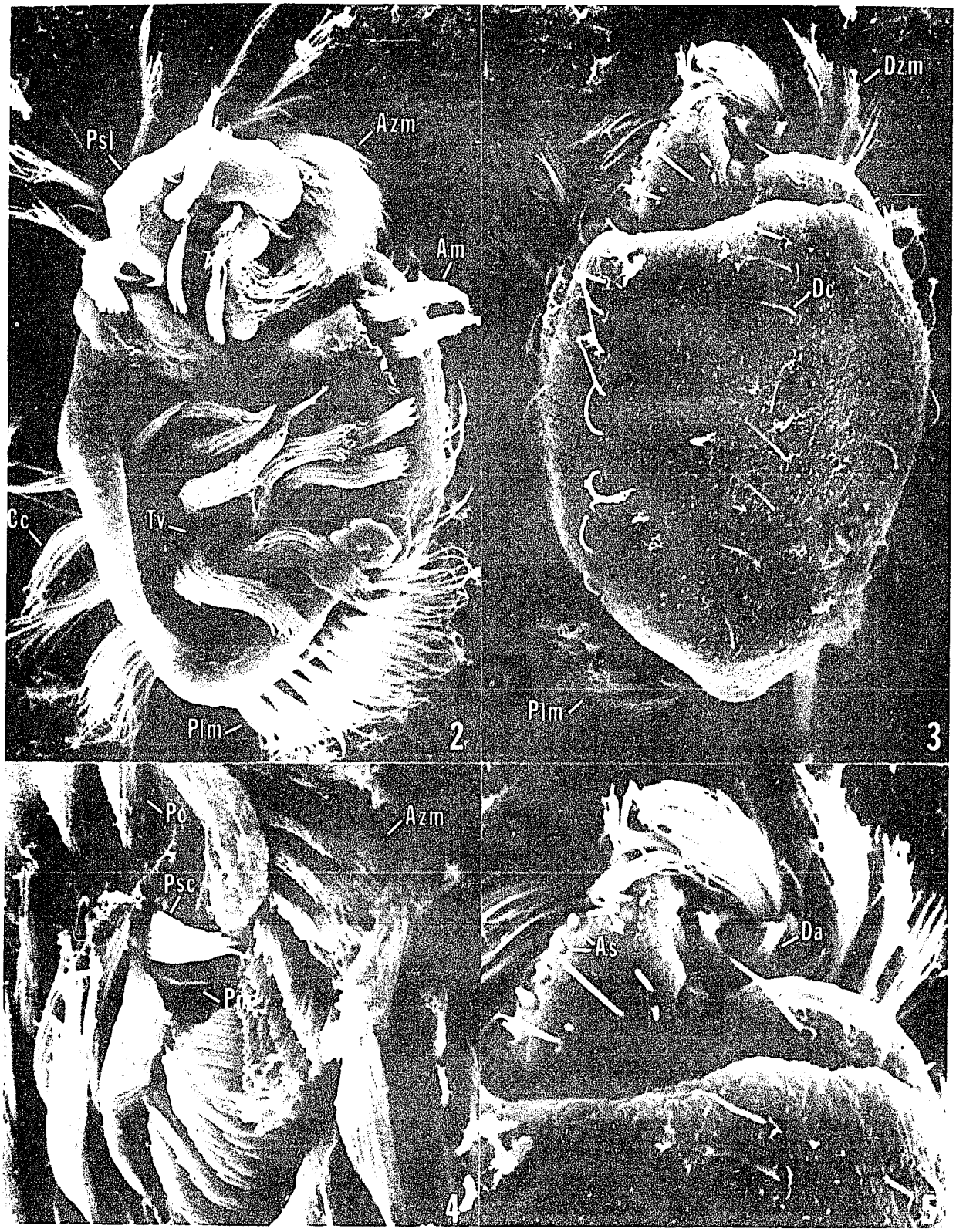


Fig. 1. Ink drawing of Discocephalus ehrenbergi DRAGESCO, 1960, based on a protargol stained specimen (ventral aspect).

Fig. 2,3. Scanning electron micrographs of non-dividing cells. Ventral aspect (fig. 2): Am--anterior marginal cirri, Azm--adoral zone of membranelles, Cc--caudal cirri, Plm--posterolateral marginal cirri, Psl--peristomial lobe, Tv--transverse cirri. Dorsal aspect (fig 3): Dc--dorsal cilium, Dzm--dorsal zone of membranelles, Plm--posterolateral marginal cirri. (X 1 600, 1 900)

Fig. 4,5. Scanning electron micrographs depicting structures of the cephalized region of the cell. Ventral aspect (fig. 4): Azm--adoral zone of membranelles, Pc--paroral cirrus, Pm--paroral membrane, Psc--peristomial cirrus. Dorsal aspect (fig. 5): As--adoral spines, Da--dorsal "antler"-like structure. (X 4 300, 3 300)

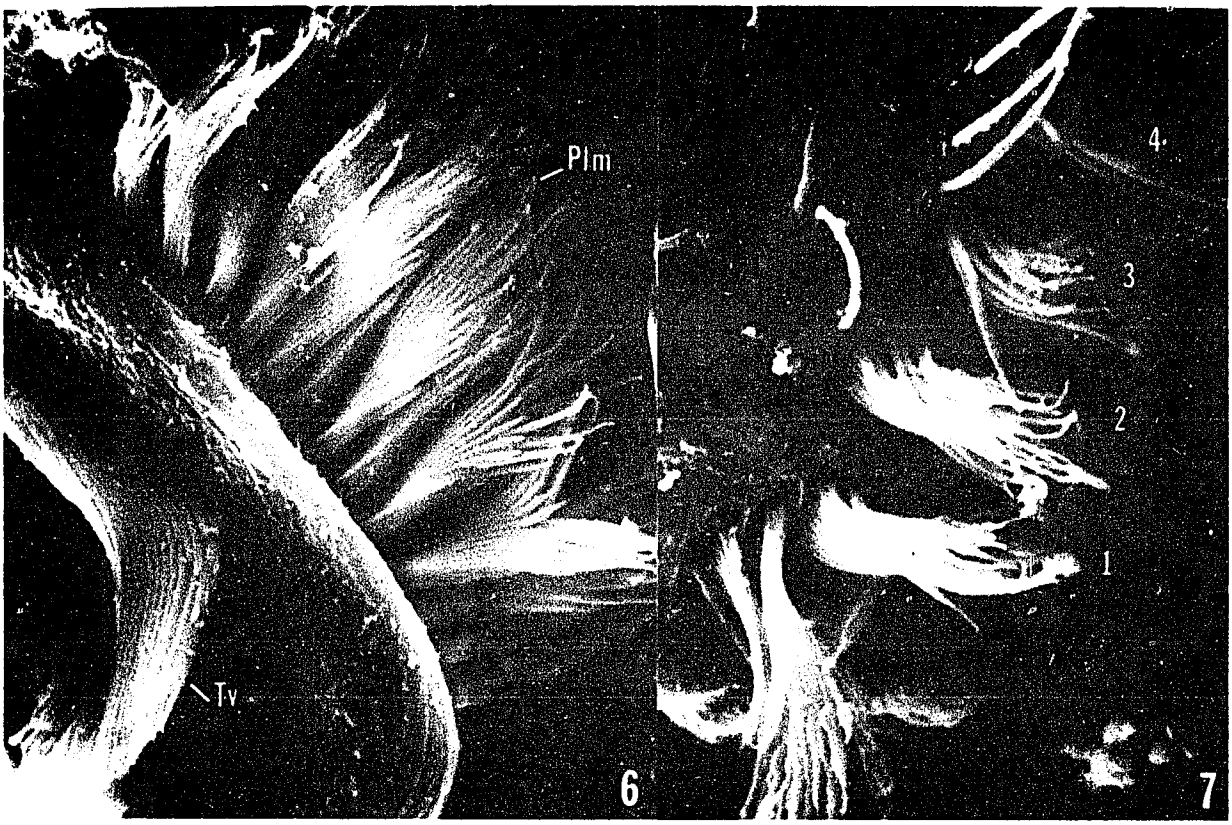

Psl
Azm
Am
Cc
Tv
Plm
2
Dzm
Dc
Plm
3
Pc
Azm
Psc
Pl
4
As
Da

Cortical spines and protrusions. On the cephalized region of the cell are found 2 kinds of dorsal, cortical protrusions: a series of 11 adoral spines extending anteriorly just above the lapel membranelles and a multipronged, antler-like structure lying just posterior to the collar membranelles (fig. 3). The adoral spines are short (1-2 μm) and pointed; each appears closely associated with the leftmost portion of a lapel membranelle (fig. 5). The dorsal antler consists of a main branch (approximately 10-15 μm) extending obliquely left and anteriorly; from the main branch extend 4 shorter (2.5 μm) branches directed dorsally and approximately 6 intermembranellar branches which run dextrally (fig. 5). Both the adoral spines and the dorsal antler are protargol positive structures (fig. 8).

Ciliature. Frontal ciliature consists of a U-shaped group of 7-10 hypertrophied transverse cirri plus 2 smaller accessory transverse cirri set within the ventral concavity, 3 right frontal cirri, 1 right anterolateral (migratory) cirrus, several cirri on the cephalized region including 2 malar and a paroral cirrus (figs. 1, 2). The paroral cirrus along with the anteriormost streak II cirrus (see below) form the 2 large anterior cirri typical of the genus.

Somatic ciliature comprises marginal cirri, caudal cirri and dorsal bristles. Left marginal cirri are divided into 2 groups: an anterior set of 2 hypertrophied cirri plus a posterolateral set of 13-14 membranelle-like cirri located within a cortical depression (figs. 2, 6). Right marginal cirri are absent. On the dorsal surface are 6 rows of long cilia (7-8 μm in length) that extend onto the cephalized region (fig. 3). Four sets of caudal cirri are present at the posterior right border of the dorsal surface: the first

Fig. 6,7. Scanning electron micrographs of the posterior region of the cell. Fig. 6: posterolateral marginal cirri (Plm) lie within a cortical depression to the left of the transverse cirri (Tv). Fig. 7: caudal cirri (numbered 1-4) are actually sets of cirri located on the right dorsal cell surface. (X 3 400, 4 400)


Plm
Tv
6
4
3
2
1
7

(posteriormost) is composed of 4 cirri; the second is composed of 3 cirri; the third is composed of 2 cirri; the fourth consists of a single cirrus (fig. 7). Although this is the arrangement of caudal cirri usually observed, individuals of some populations possess only 3 sets of caudal cirri (table 1). These sets are arranged in a series (from posterior to anterior) of 3, 2, 1 or 3, 3, 2.

Buccal ciliature comprises approximately 18 lapel (ventral) membranelles, approximately 6 collar (dorsal) membranelles (with a distinctly longer cilia) and a paroral and endoral membrane. The paroral membrane consists of 2 rows of cilia that emerge from below the right buccal overture (the left border of the peristomial lobe), extending anteriorly to a buccal cleft. Here the paroral membrane differentiates into a tongue-like ciliary organelle, the peristomial cirrus (figs. 2, 4). The deeper endoral membrane is a single row of cilia.

Ultrastructure

Cytoskeleton. The cytoskeleton of D. ehrenbergi is extensive and complex (the major components of this system are depicted in fig. 8; see also DRAGESCO, 1965). There are 2 separate parts to the cytoskeleton: a posterior portion, dominated by a large central microtubular trunk and an anterior portion within the cephalized region of the cell. The posterior cytoskeleton includes microtubules originating on the peripheral fibrillar matrix of cirri (figs. 9-11). The central cytoskeletal trunk is formed by the union of anterior microtubular bundles from each transverse cirrus (fig. 12). Branches from the anterior cytoskeleton extend to frontal cirri, collar membranelles, the dorsal antler, and both membranes of the paroral

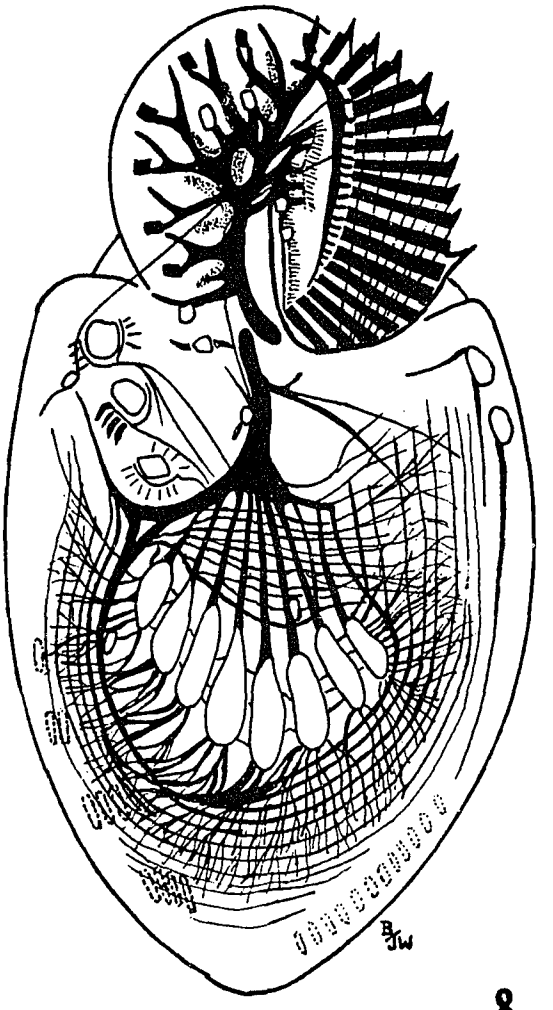


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Fig. 8. Ink drawing based on protargol stained specimens of D. ehrenbergi depicting major components of the cytoskeleton. Stippled areas represent dorsal structures.

Fig. 9,10,11,12. Transmission electron micrographs of frontal cirri. Microtubular bundles (Mb) originate on the peripheral fibrillar matrix (Fm) of each cirrus. Postciliary microtubules (Pmt) are associated with the posterior kinetosomes while transverse microtubules (T) are associated with the anterior kinetosomes of each cirrus; single transverse microtubules (arrows) are associated with internal cirral kinetosomes. A cytoskeletal trunk (Ct) is formed by the union of anterior microtubular bundles (Amb) from each transverse cirrus. (X 32 500, 30 000, 23 500, 19 100)

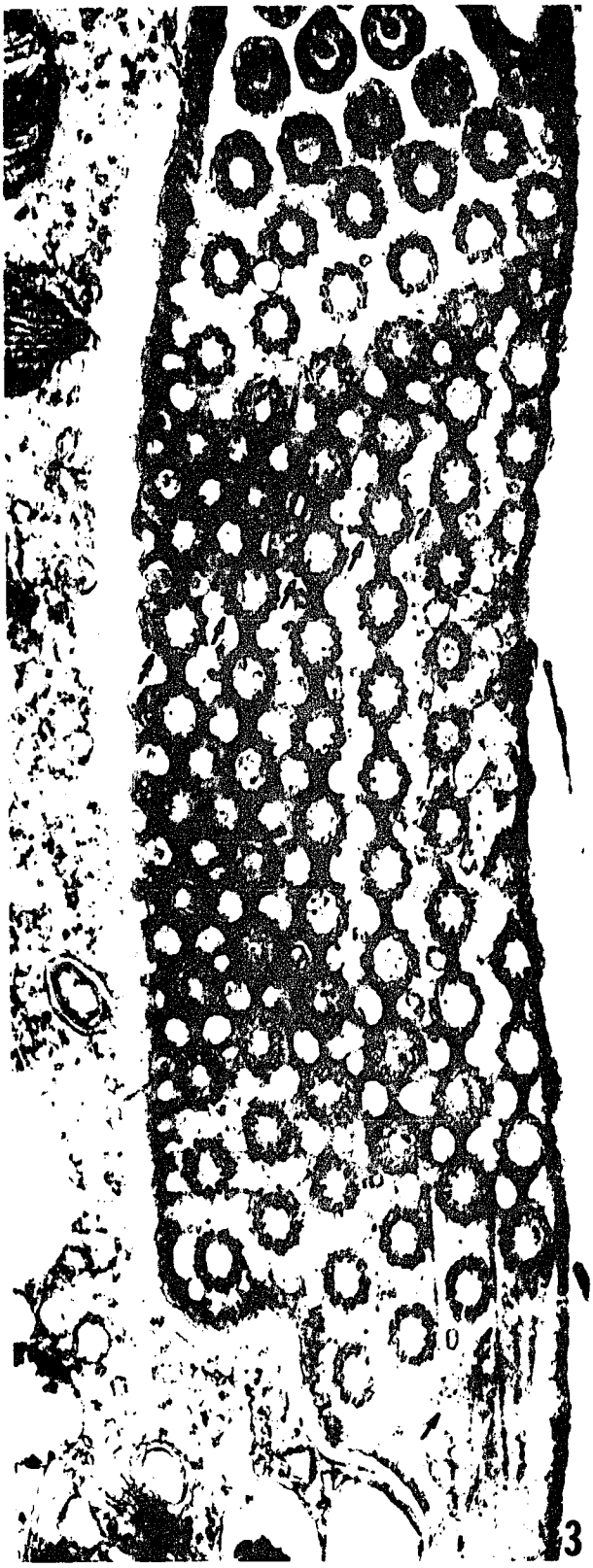

fm
Mb
t
Mb
Pmt
Pmt
9
10
T
Ct
Amb
11
12

apparatus. Small connectors extend from a paroral branch to attach to the right base of each lapel membranelle; on the left of each lapel membranelle are the adoral spines. Thin rootlets (submembranellar fibers) descend posteriorly into the cytoplasm from each membranelle at the region of the adoral spine. A postmembranellar fiber runs posteriorly from near the adoral spines to subtend the proximal membranelles, then curves anteriorly to join with the paroral branch of the cytoskeleton. All components of the cytoskeleton in D. ehrenbergi are composed of microtubules.

Cirri. The cirral bases of D. ehrenbergi are variously shaped packets of kinetosomes ranging from parallelogram arranged sets to irregular polygon shaped sets (figs. 9-11). A fibrillar sheath surrounds each cirrus distally near the cell surface; it then descends to encircle the kinetosomes proximally as the peripheral cirral matrix. Nine interkinetosomal connectives (continuous with the peripheral fibrillar matrix) join neighboring kinetosomes: a right anterior (RA), left anterior (LA), left oblique (LO), left (L), left posterior (LP), posterior (P), right posterior (RP), right (R), and right oblique (RO). Connectives LO, RO and P anastomize between kinetosomes forming a recticulate pattern that is repeated throughout the cirral base. Two additional connectors, left and right postkinetosomal connectors, located on each side of the postciliary microtubules, link each posteriormost cirral kinetosome with the peripheral fibrillar matrix (figs. 9-11).

Cirral kinetosomes can be as few as 12 in some frontal cirri, to greater than 100 in transverse cirri. Rows of transverse cirri kinetosomes are oriented at approximiately 40^{o} to the longitudinal

Fig. 13. Transmission electron micrograph of a transverse cirrus. Arrows indicate single postciliary microtubules associated with internal cirral kinetosomes. (X 37 100)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axis of the cell (fig. 13), while in other frontal cirri the angle is increased to nearly 100° (fig. 9). This kinetosomal alignment (ranging from acute to obtuse arrays) can be explained by the degree of rotation of procirri after initial streak formation during morphogenesis (described below).

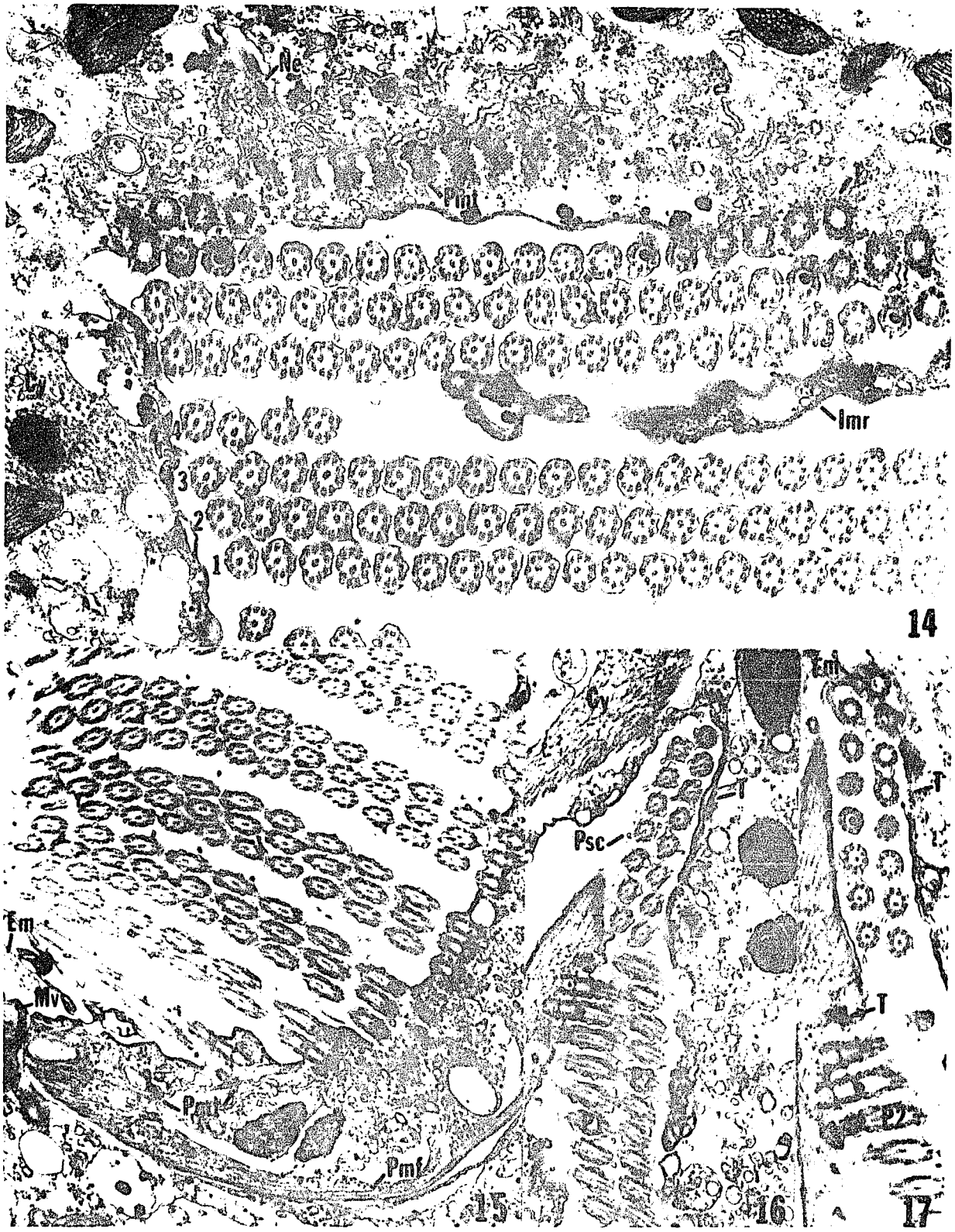
Transverse microtubular ribbons arise from near triplet number 4 of kinetosomes bordering the anterior left edge of each cirrus; these ribbons appear to extend directly toward the pellicle (figs. 10, 11). Postciliary microtubular ribbons originate from the posteriormost cirral kinetosomes, then extend ventrally and posteriorly, presumably to contribute to a posterior microtubular bundle (figs. 9, 10). In addition to these complete microtubular ribbons, single microtubules are present at triplet number 9 (a remnant postciliary microtubular ribbon) of internal cirral kinetosomes (figs. 9, 10). The internal postciliary microtubules of transverse cirri extend posteriorly between kinetosomes to contribute to a posterior microtubular bundle (fig. 13).

Buccal apparatus. The membranelles are paramembranelles; each comprises 4 rows of kinetosomes: the first (posteriormost) and second are of equal length and are the longest rows, the third row is shorter (having 4-5 fewer kinetosomes than rows 1 and 2), and the fourth row (anteriormost) is shortest with only 3-4 kinetosomes per row (fig. 14). Membranelles of the lapel region have more kinetosomes per row than proximal or distal membranelles.

Transverse microtubular ribbons are associated with triplet 4 of each kinetosome of row 4 and those kinetosomes of row 3 that are not bordered by row 4 kinetosomes (fig. 14). Postciliary microtubu-

Fig. 14,15. Transmission electron micrographs of the adoral membranelles. Each membranelle comprises 4 kinetosomal rows (fig. 14): row 1 (posteriormost) and row 2 are longest, row 3 is shorter, and row 4 is shortest. Nematodesmal microtubules (Ne) extend from the base of membranellar kinetosomes. Postciliary microtubules (Pmt) are associated with row 1 kinetosomes; these extend along the intermembranellar ridge (Mr) joining with postciliary microtubules of adjacent membranelles (fig. 15) to form a postmembranellar fiber (Pmf). Membrane vesicles (Mv) line the right buccal cavity beside the endoral membrane (Em). A portion of the microtubular cytoskeletal (Cy) is also evident. (X 21 400, 12 800)

Fig. 16,17. Transmission electron micrographs of the paroral apparatus. The paroral membrane consists of 2 rows of kinetosomes, P1 and P2: the anterior part of the paroral membrane is differentiated into the peristomial cirrus (Psc). The peristomial cirrus extends ventrally from the buccal cleft and is surrounded by a fibrillar matrix (Fm) whereas the paroral membrane extends laterally and lacks a fibrillar matrix. Transverse microtubules (T) and part of the cytoskeleton (Cy) are visible. (X 13 800, 17 500)


Ne
Pmf
Imr
3
2
1
14
Cy
Psc
Em
T
Em
Mvb
Pmf
Pmf
15
16
17

lar ribbons arise from only row 1 membranellar kinetosomes; these ribbons course laterally within the intermembranellar ridge to contribute to the formation of the postmembranellar fiber that extends along the left and posterior borders of the zone of membranelles (figs. 14, 15). Nematodesmal microtubules descend from the base of membranellar kinetosomes into the cytoplasm where they coalesce with other components of the microtubular cytoskeleton (fig. 14).

The paroral membrane is a longitudinal row of kinetosomal pairs (polystichomonade); this membrane lies just below the right buccal overture, extends anteriorly, then ends at the buccal cleft (figs. 4, 16, 17). It is the shorter of the 2 oral membranes. Within the buccal cleft, the anterior end of the paroral membrane is differentiated into a separate unit: the short (7-8 kinetosomal pairs) peristomial cirrus observed in S.E.M. (figs. 4, 16, 17). Whereas the posterior part of the paroral membrane protrudes laterally from the right buccal wall, the peristomial cirrus extends dorsoventrally from the buccal cleft (figs. 16, 17). Furthermore, the posterior paroral row is membrane-like (without a peripheral fibrillar matrix), while the differentiated anterior segment is surrounded by electron dense material - hence appearing more cirrus-like (fig. 17).

Postciliary microtubules are present on the right membrane border; transverse microtubules are present on the left border of the paroral membrane (fig. 17). Transverse microtubular ribbons are also associated with the left kinetosomes of the peristomial cirrus; these microtubules extend directly toward the pellicle (figs. 16, 17).

The longer, single row of kinetosomes of the endoral membrane is deep within the buccal cavity. It arises dorsal to the paroral

membrane, extending from just posterior of the peristomial cirrus, along the buccal wall to the level of the proximalmost membranelle (well beyond the posterior extent of the paroral membrane) (fig. 8). This membrane (a stichomonade) protrudes dorsally from the ventral wall of the buccal cavity--a position which results in the endoral kinetosomes being oriented at 180° relative to the kinetosomes of the paroral membrane. Thus, each endoral kinetosome appears as a clockwise cartwheel (as viewed from outside the cell). This rotation does not affect the position of postciliary and transverse microtubular ribbons: postciliary microtubules originate from the right, transverse microtubules arise from the left of each endoral kinetosome (fig. 18). The transverse microtubules radiate from each kinetosome to anastomose with other components of the cytoskeleton. The postciliary microtubules are directed posteriorly in an overlapping series. Packets of membrane (pharyngeal discs) line the right side of the buccal cavity near the cytostome (fig. 15).

Cortical spines. The adoral spines and dorsal antler observed in S.E.M. are membrane-bound bundles of microtubules that are continuous with the anterior cytoskeleton and, in the antler, the nematodesmal microtubules of the paramembranelles (fig. 19). Most microtubules comprising the dorsal antler are arranged linearly along the longitudinal axis of the structure. Some microtubules, especially those located beside the antler membrane, are arranged perpendicularly to the longitudinal axis of the structure (fig. 20).

Dorsal bristle complex. Each of the dorsal bristle rows of D. ehrenbergi consist of a series of kinetosomal pairs - each pair is positioned 60° to the longitudinal axis of the cell. The anterior

Fig. 18. Transmission electron micrograph of the endoral membrane; postciliary microtubules (Pmt) arise from the right and transverse microtubules (T) arise from the left of endoral kinetosomes. (X 35 400)

Fig. 19,20. Transmission electron micrographs of the dorsal "antler"-like structure (Da) between dorsal membranelles (Dm), fig. 19, as well as protruding from the cortex, fig. 20. Nematodesmal microtubules (Ne) from dorsal membranelles join microtubules of the "antler"; both then contribute to the cytoskeleton of the cephalized region. (X 18 500, 29 600)

Fig. 21. Transmission electron micrograph of a sagittally sectioned, dorsal bristle complex. Each bristle complex lies within a cortical pit; the alveolar membranes (Av) extend only to the pit wall. The anterior kinetosome is ciliated, the posterior is nonciliated. Transverse microtubules (T) are associated with the anterior kinetosomes while postciliary microtubules are associated with the posterior kinetosome. Nematodesmal microtubules (Ne) and a fibrillar rib (Fr) are evident. (X 44 700)


Pmt
18
Dm
Da
Dm
19


20
Av
Tr
Pmt
Ne
21

kinetosome of most doublets bear a long cilium (7 μm), the posterior kinetosome is non-ciliated (figs. 21, 22). In some bristle complexes on the cephalized region of the cell, however, the anterior kinetosme bears a short (2-3 μm) cilium (fig. 5). Both kinetosomes lie within a cortical pit; whereas the cell membrane extends into the pit and onto the cilium, the alveolar membrane extends only to the pit wall (fig. 21). Alveolar plates, as observed in Euplotes (HAUSMANN and KAISER, 1979) and Certesia (WICKLOW, unpublished), are absent.

Just below the cell membrane a band of dense fibrillar material surrounds the distal portion of each kinetosomal pair (figs. 23, 24). From this distal band descend a series of fibrillar ribs that curve proximally toward the kinetosomes where they join a second band (fig. 25). Radiating fibrillar arms then link this second band to the kinetosomal triplets, thereby completing a basket-like framework around each bristle pair (fig. 25). At the anterior end of this basket, near the cell surface, is a small outpocket (fig. 24).

Two desmoses connect the kinetosomes of each pair: a thick left desmose between triplet 9 of the anterior kinetosome and triplets 4 and 5 of the posterior kinetosome, and a thin right desmose between triplet 2 of the anterior kinetosome and triplet 3 of the posterior kinetosome (fig. 26). The left side of each bristle complex is bordered by a dense mass of fibrillar material; nematodesmal microtubules originate from this fibrillar patch as well as from the base of the bristle kinetosomes (fig. 26). Although some nematodesmal microtubules run posteriorly, most radiate toward the left side of the cell (fig. 27).

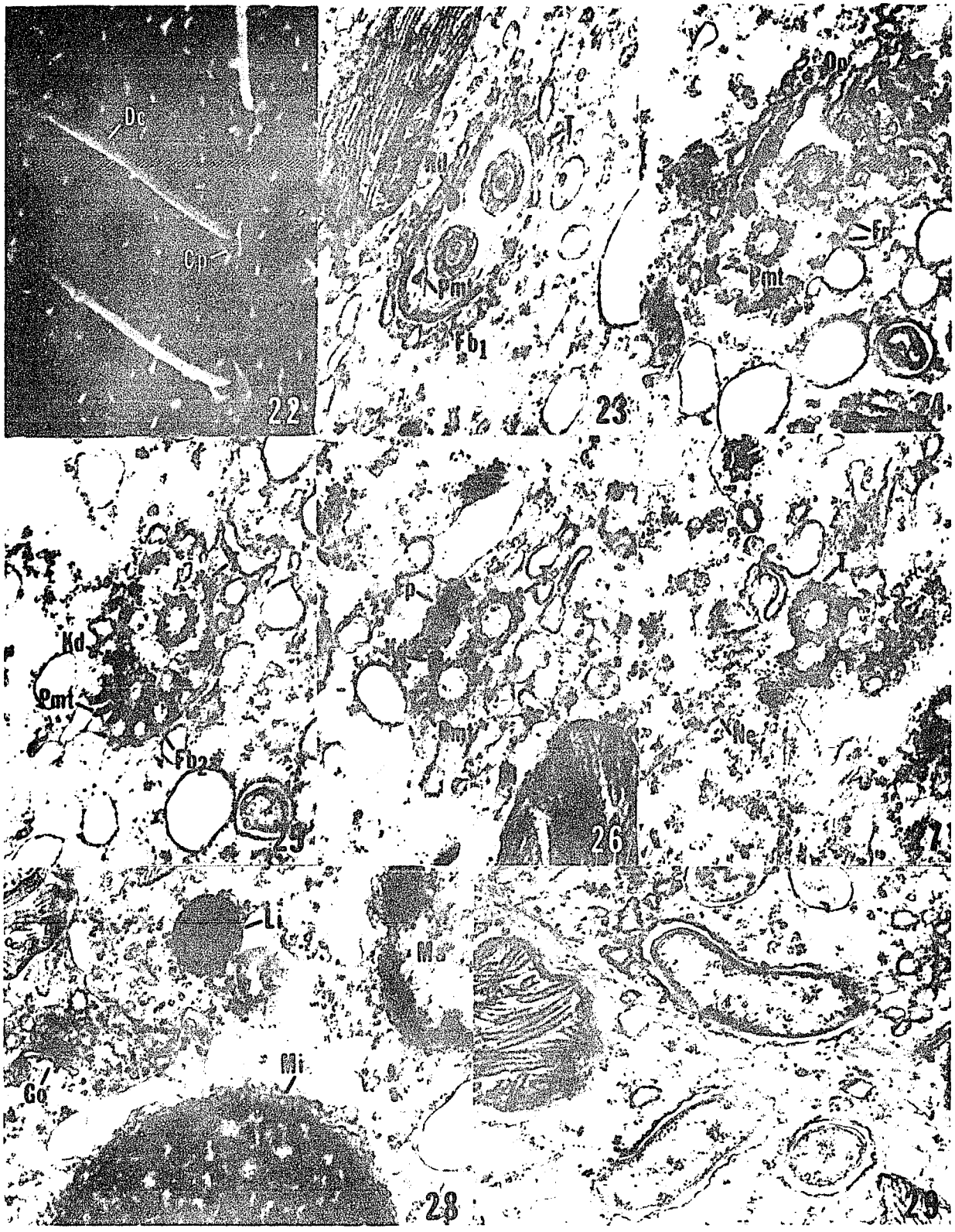
A transverse microtubular ribbon, consisting of 5 microtubules,

Fig. 22. Scanning electron micrograph of a dorsal cilium (Dc) extending from a cortical pit (Cp). (X 5 500)

Fig. 23,24,25,26,27. Transmission electron micrographs of a series of cross sectioned, dorsal bristle complexes. A fibrillar band (Fb1) surrounds each complex distally; fibrillar ribs (Fr) descend from Fb1 to a second fibrillar band (Fb2). Fibrillar connections join Fb2 with the base of bristle kintosomes. A fibrillar patch (F) borders the left side of the complex. Postciliary microtubules (Pmt) and a kinetodesmal fiber (Kd) are associated with the posterior kinetosome, while transverse microtubules (T) and a single postciliary microtubule (fig. 26) are associated with the anterior kinetosome of the complex. Nematodesmal microtubules (Ne) descend from bristle kinetosomes and the fibrillar patch. A small outpocketing (Op) extends from the anterior of the bristle pit wall. (X 35 600, 38 000, 38 600, 37 500, 35 600)

Fig. 28. Transmission electron micrograph of the cytoplasm showing a macronucleus (Ma), micronucleus (Mi), lipid inclusion (Li), and Golgi complex (Gc). (X 28 600)

Fig. 29. Transmission electron micrograph of bacteria-like endosymbionts. (X 33 500)


Dc
Cp
22
Pmt
Fb1
23
Dp
Fb
Pmt
24
Nd
Pmt
Fb2
Pmt
Ne
26
Li
Ms
Mi
Go
28
28

originates at triplets 4 and 5 of the anterior kinetosome. Two postciliary microtubular ribbons are associated with each bristle complex: a single postciliary microtubule originates near triplet 9 of the anterior kinetosome and a series of 3 postciliary microtubules arise from triplet 9 of the posterior kinetosome. Both transverse and postciliary microtubular ribbons extend dorsally toward the pellicle. A kinetodesmal fiber is associated with triplets 6 and 7 of the posterior kinetosome; it runs slightly anteriorly at a sharp angle toward the pellicle (figs. 23-27).

Cytoplasm. Within the ribosome rich cytoplasm are scattered over 100 macronuclei. Fewer micronuclei, containing dense chromatin material, are also present as well as vesicles of the Golgi complex and dense lipid inclusions (fig. 28).

Groups of rod-shaped endosymbiotic bacteria (approximately 1.3 μm in length, approximately 0.3 μm in width) occur throughout the cytoplasm. Two membrane systems surround each symbiont: an outer membrane (or cell wall) and an inner cytoplasmic membrane (fig. 29).

Morphogenesis

Cortical morphogenesis during cell division in D. ehrenbergi occurs in one latitudinal zone; from this zone develop buccal, frontal, and somatic ciliature for both proter and opisthe daughter cells (fig. 30a-i). The first morphogenetic event observed within this zone is the formation of an oral primordium (OP) at the cell surface. Later, in close association with the enlarging OP, an additional lacelike network of primordia arises: the frontal primordia (FP) (subsequently developing into an oblique series of 7-9 frontal streaks) and, beside the right edge of the OP, an undulating membrane primordium

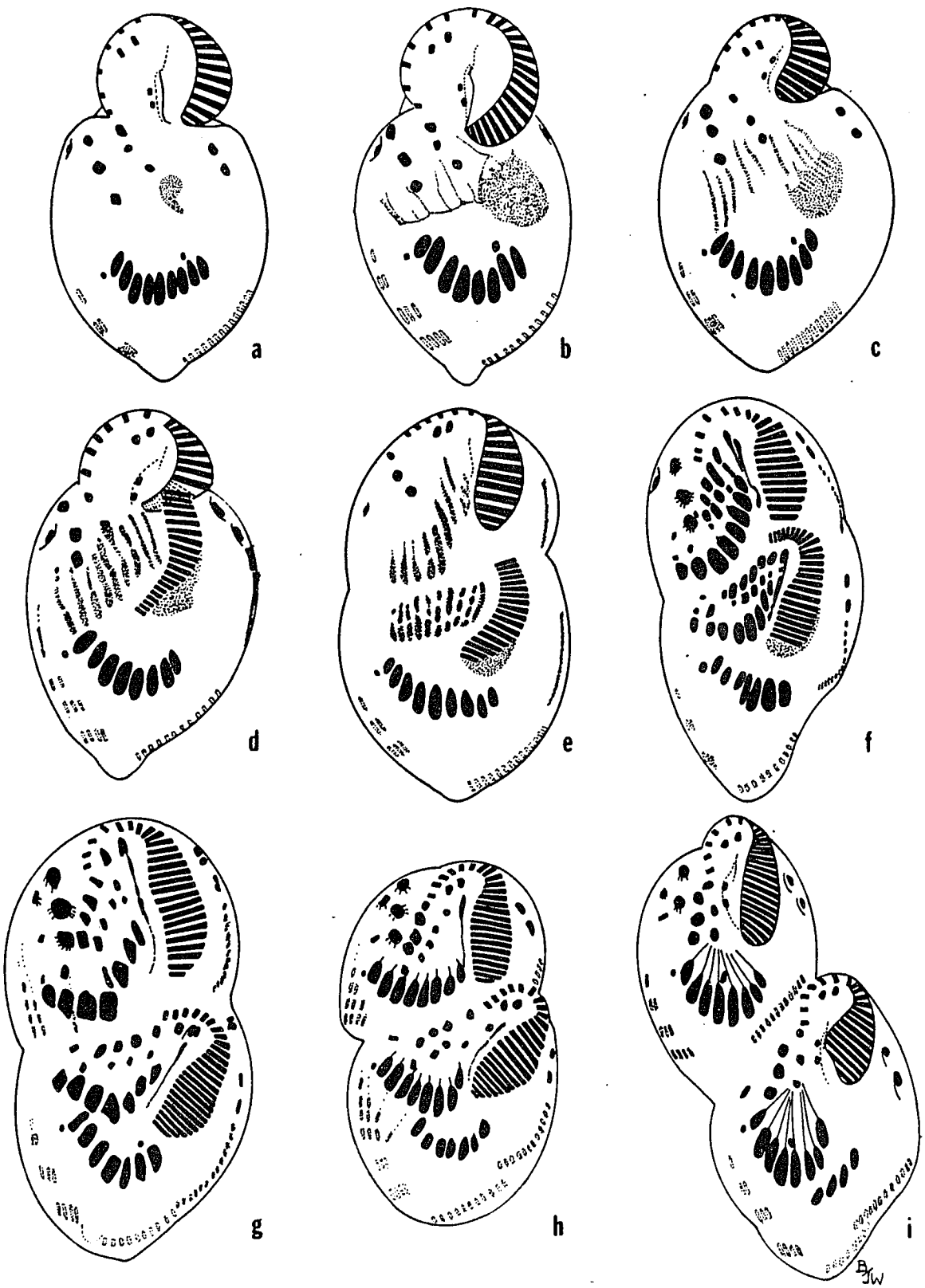


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Fig. 30. A sequence of line diagrams, based on protargol stained specimens of ventral, cortical, morphogenetic stages during cell division. Black areas represent ciliary organelles.

Fig. 31,32,33. Scanning electron micrographs depicting cortical morphogenesis. Oral (Op) and frontal (Fp) primordia develop on the ventral surface (fig. 31,32). Frontal primordia appear as a series of oblique streaks within cortical grooves. A paroral primordium (P) is separated from the Op by a cortical ridge. The marginal cirral primordium (Mcp) arises just posterior to the anterior marginal cirri. Caudal cirri are 4 sets of cirri (numbered 1-4) that develop just anterior to the parental caudal cirri, from the rightmost dorsal kineties. (X 2 400, 4 400, 2 700)


Fp
Op
31
Fp
P
Op
Mcp
32
1
2
3
4
Pcc
33

(UMP). From the UMP differentiate the paroral and endoral membranes; a paroral cirrus develops from the anterior end of the paroral membrane.

While the OP differentiates membranelles (in a posteriad direction), a cortical invagination (the future buccal cavity) is formed with the membranelles and endoral membrane within, the paroral membrane along the right edge. A cortical ridge between the membranelles and the paroral membrane represents the future right buccal overture (fig. 31, 32).

Meanwhile, the frontal streaks, developing within cortical grooves (fig. 32), split into proter and opisthe frontal fields (fig. 30c-e) and the parental paroral apparatus begins to dedifferentiate. Four oblique ranks of procirri differentiate from each of these frontal fields: transverse cirri compose the first (posteriormost) rank; 2 accessory transverse cirri compose the second rank; 1 migratory, 3 midfrontal and 2 malar cirri compose the third rank; 3 right frontal cirri and 1 anterior frontal cirrus compose the fourth rank (fig. 30f). At the same time as the collar membranelles are curving toward the cell's right, the frontal cirri move anteriorly, also curving to the right, until they are disposed in a longitudinal series (fig. 30f-i). The migratory cirrus is positioned on the right lateral surface, just posterior to the distal membranelles. Only the transverse cirri appear to move posteriorly as they assume a U-shaped configuration. Microtubules are formed at the anterior edge of each transverse cirrus; these grow anteriorly, then eventually enlarge and unite to form the major component of the posterior cytoskeleton (fig. 30h,i).

When the frontal streaks are still in a single field and the membranelles have begun to differentiate within a cortical invagination,

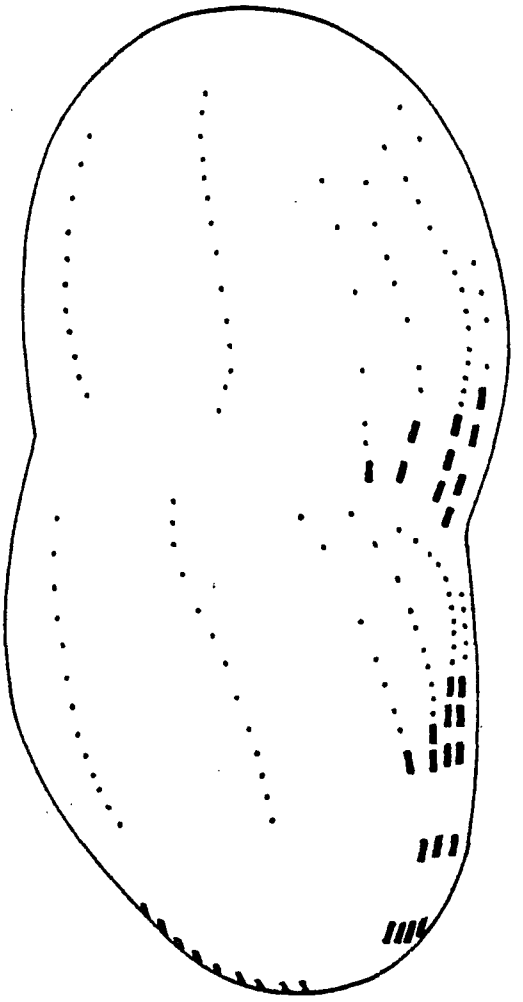


Fig. 34. Line diagram, based on a protargol stained specimen, showing the origin of caudal cirri (black rectangles) from the rightmost dorsal kineties (black dots) during cell division.

a left marginal cirral primordium (MP) appears (figs. 30d, 31). It arises in close association with the posterior marginal cirrus, proliferates posteriorly, then divides into anterior (proter) and posterior (opisthe) marginal anlagen. From each of these MP streaks differentiate 2 kinds of cirri: the pair of hypertrophied anterior cirri, and the posterolateral series of membranelle-like cirri. Although originating from the same within-row primordium, these 2 sets of cirri differ in position, kinetosomal arrangement, and presumably, function.

The six dorsal kineties arise by within-row development. In addition to dorsal bristles, caudal cirri differentiate from the 4 rightmost dorsal bristle primordia. The 4 caudal cirri are actually 4 sets of cirri. The first (posteriormost) set comprises 4 cirri - one from each of the 4 rightmost kineties; the second set consists of 3 cirri from the 3 rightmost kineties; the third set consists of 2 cirri from the 2 rightmost kineties; the fourth is a single cirrus arising from the rightmost kinety (figs. 33, 34).

The parental ciliature begins to be disassembled and resorbed during late developemnt, after cytokinesis is underway. Only the parental membranelles are retained (partially redifferentiated) by the proter.

Discocephalus rotatorius HEMPRICH and EHRENBERG 1831

D. rotatorius, discovered by HEMPRICH and EHRENBERG, has been observed and described by SAUERBREY (1929), FAURÉ-FREMIET (1951), DRAGESCO (1960), HARTWIG and PARKER (1977). This species shares many characters with D. ehrenbergi: general size, shape, cirral arrangement, presence of only 2 anterior left marginal cirri, absence of

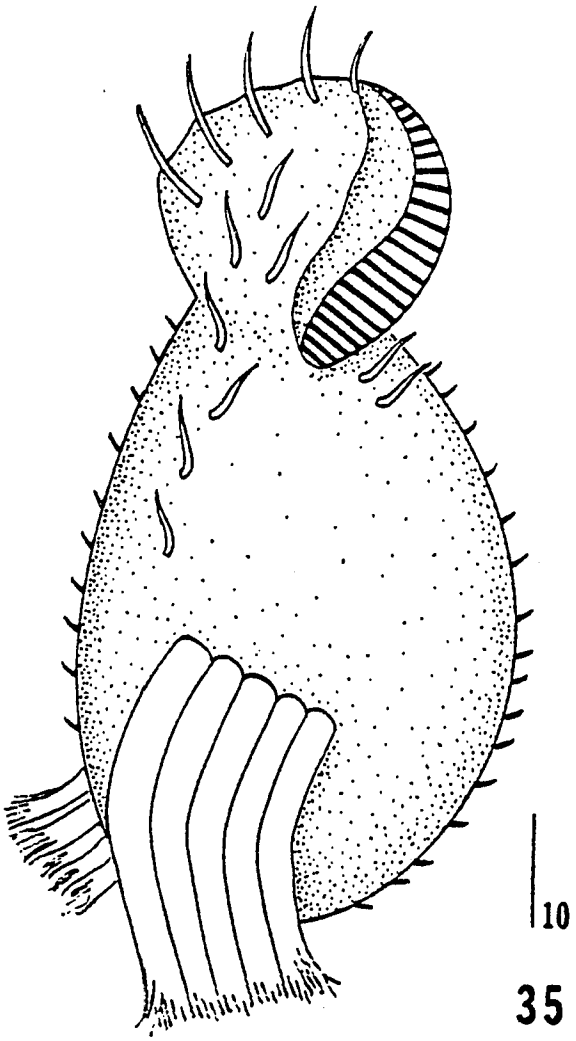


Fig. 35. Ink drawing of Discocephalus rotatorius HEMPRICH and EHRENBERG, 1831 (after SAUERBREY, 1928).

right marginal cirri, as well as the peculiar behavior of ingesting sand particles. SAUERBREY (1929) depicted 8 frontal cirri (fig. 35) on the cephalized region of D. rotatorius (see also DRAGESCO, 1960); the anteriormost 5 cirri in this group, however, probably represent collar membranelles. Two midfrontal, 2 right frontal, and only 5 transverse cirri are present - accessory transverse cirri are absent. Twelve to twenty posterolateral marginal cirri are present as well as 3-4 caudal cirri. Although observed many times, this species needs redescription.

Marginotricha grandis (DRAGESCO, 1954) JANKOWSKI 1978

M. grandis is a large (200 µm) cephalized hypotrich possessing 4 large anterior frontal cirri, a midfrontal row (disposed toward the right) consisting of approximately 45 cirri, a group of 10 transverse cirri, and 2 accessory transverse cirri (fig. 36). Both right and left marginal cirral rows are present; left marginal cirri are divided into an anterior row of approximately 46 cirri and a posterolateral group of approximately 13 cirri. Two posterior right cirri probably represent caudal cirri. Membranelles extend only to the anterior of the cell. Numerous macronuclei are present.

Prodiscocephalus minimus (DRAGESCO, 1968) JANKOWSKI 1979

In 1968 DRAGESCO described an additional species of Discocephalus from sands of Arcachon: D. minimus (fig. 37). This species is cephalized, possesses right and left maringal cirri (the left marginal cirral row is differentiated into an anterior set of 7-8 cirri and a posterior set of 9-10 cirri), 2 midfrontal cirri, and 7 transverse cirri. Four additional cirri, posterior to the 4-6 right marginal

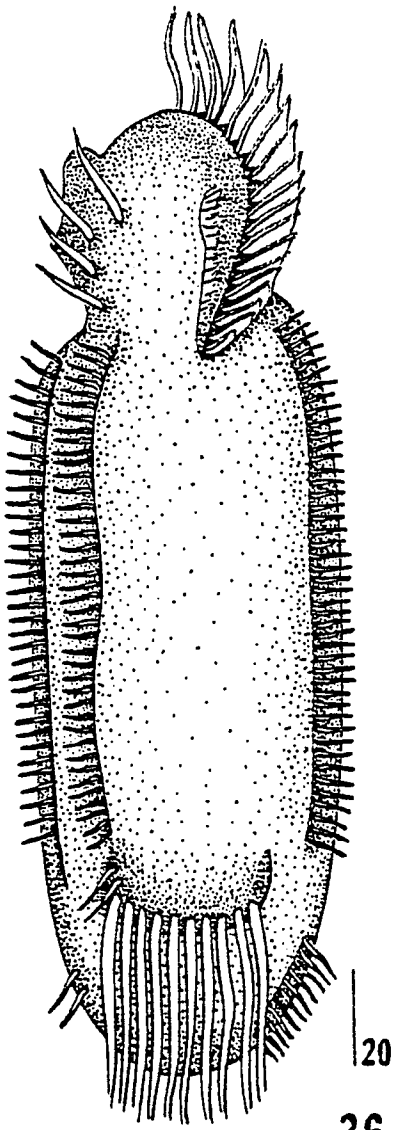


Fig. 36. Ink drawing of Marginotricha grandis (DRAGESCO, 1954) JANKOWSKI, 1978 (after DRAGESCO, 1954).

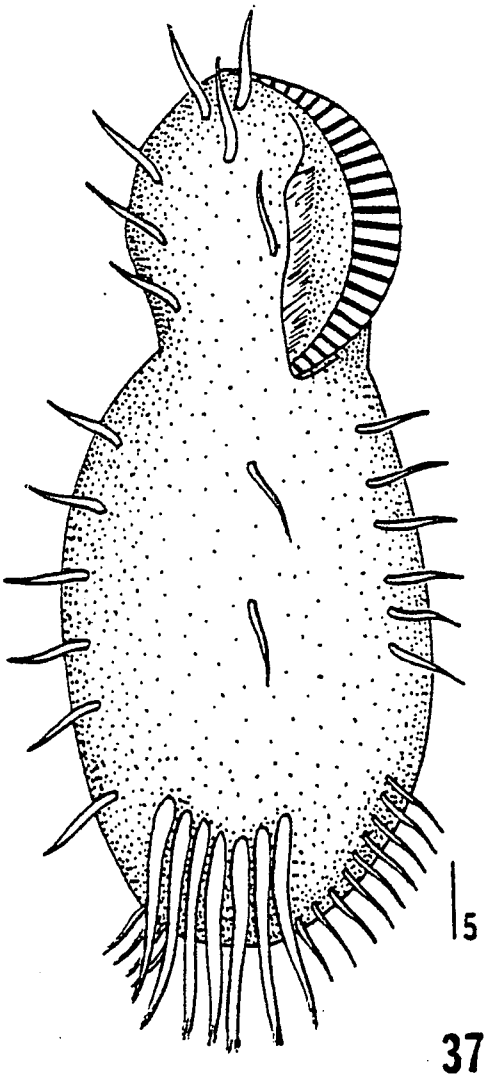


Fig. 37. Ink drawing of Prodiscocephalus minimus (DRAGESCO, 1968) JANKOWSKI, 1979 (after DRAGESCO, 1968).

cirri probably represent groups of caudal cirri. Seven addtional frontal (one probably a paroral cirrus) are present in the cephalized region. Membranelles extend only to the anterior of the cell. Three to fifteen spherical macronuclei are scattered throughout the cytoplasm.

Psammocephalus borrori n.gen., n.sp.

I discovered P. borrori (fig. 38) in the upper 3 cm of sand at Foss Beach, N.H. (type locality: 43°0'24" lat., 70°44'30" long.). This species is highly thigmotactic, but can also spiral rapidly backward when disturbed. It feeds on diatoms and algae, gliding over sand grains in a forward, then erratic back and forth motion. The name Psammocephalus is derived from the Greek roots Psammo, meaning sand, and cephala, meaning head; the species borrori is named in honor of Dr. Arthur C. Borror.

Morphology

The ciliate is approximately 100 μm in length, 35 μm in width. The posterior portion of the cell is elongate and rigid; the anterior portion is strongly cephalized and can be flexed. Like D. ehrenbergi the cephalized region forms a ventral peristomial lobe and houses the membranelles (approximately 8 collar and 20-24 lapel membranelles) and oral membranes including the paroral cirrus and a peristomial cirrus at the anterior end of the paroral membrane. In the cell's posterior region there is a midventral groove that leads posteriorly to a ventral concavity from which 5-9 transverse cirri and 2 accessory transverse cirri emerge. The remaining frontal cirri arise from the midventral groove, beside the lapel membranelles (malar cirri), and on the cephalized region. Two migratory cirri are located just posterior to the distal collar membranelles.

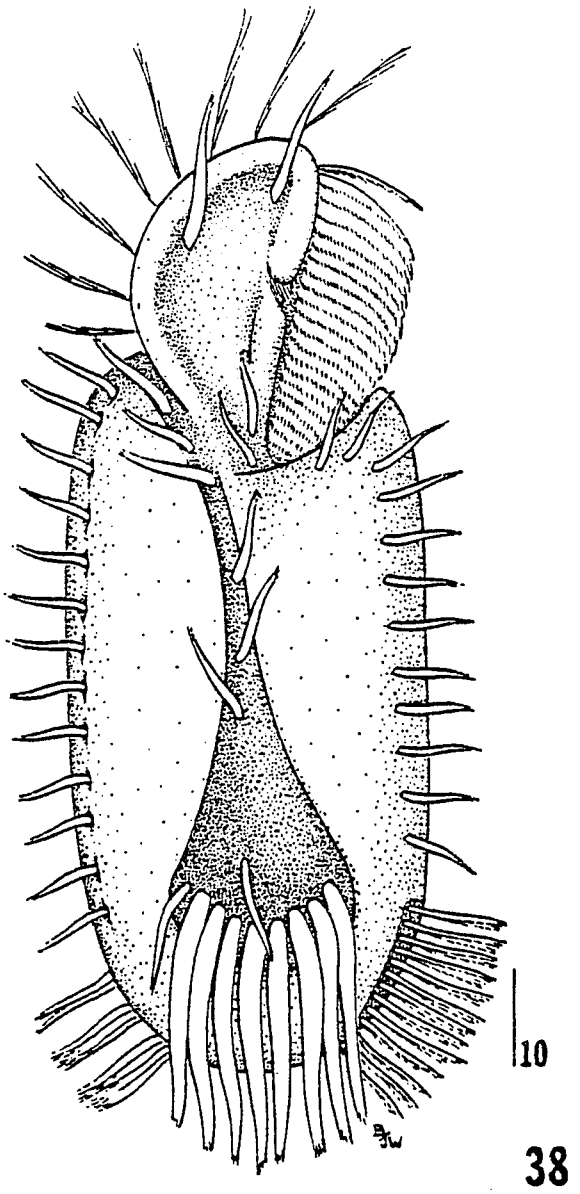


Fig. 38. Ink drawing of Psammocephalus borrori n.gen., n.sp.

As in D. ehrenbergi, left marginal cirri consist of 2 distinct groups: an anterior row of 8-24 cirri, each set ventrally at a 60° angle to the longitudinal axis of the cell, and posterolateral group of 8-13 cirri set within a cortical groove. In addition to left marginal cirri, P. borrori (unlike D. ehrenbergi) possesses right marginal cirri - a uniform row of 11-13 cirri.

On the dorsal surface there are 6 bristle rows. Five of these rows, numbers 1, 2, 4, 5 and 6 (numbering from left to right), possess extremely long cilia (7-8 μm in length); one row, number 3, possesses short cilia (approximately 2 μm in length) (fig. 39). Three sets of caudal cirri lie along the right posterior edge of the dorsal surface. The first (posteriormost) is a set of 4 cirri, the second set of 2 cirri, and the third is a set of 2 cirri.

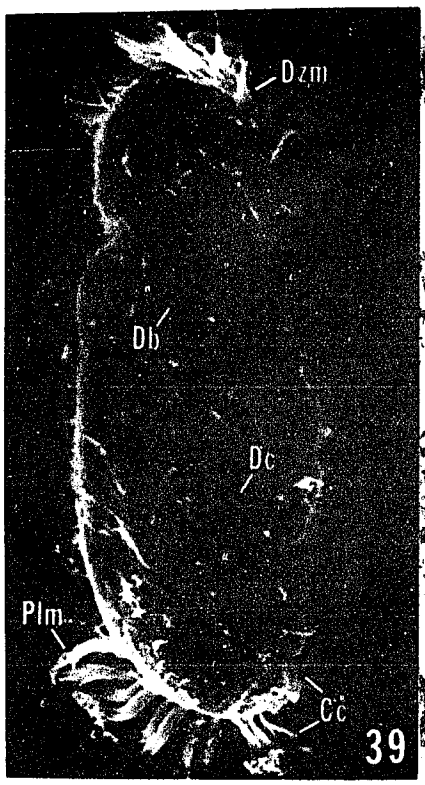
Microtubular bundles originate at the anterior edge of each transverse cirrus, extend anteriorly, then unite to form a microtubular trunk - tion has the major component of the posterior cytoskeleton. The transverse cirral kinetosomes are arranged in oblique rows; as in D. ehrenbergi, each internal kinetosome bears a single postciliary microtubule that runs posteriorly between kinetosomal arrays (fig. 40). Large bundles of nematodesmal microtubules descend from the cirral base, then extend posteriorly to join with the U-shaped, post-transverse element of the cytoskeleton (fig. 40). The cytoskeleton of the cephalized region is less complex than that of D. ehrenbergi and microtubular protrusions are absent. The cell is multimacronucleate with up to 120 macronuclei.

Morphogenesis

I observed only an incomplete series of reorganization morphogenesis

Fig. 39. Scanning electron micrograph of Psammocephalus borrori (dorsal aspect). Whereas most dorsal kineties possess long (7 μm) cilia (Dc), row 3 possess short (2 μm) bristles (Db). Dorsal membranelles (Dzm), posterolateral marginal cirri (Plm), and caudal cirri (Cc) are also evident. (X 1 200)

Fig. 40. Transmission electron micrograph of the transverse cirri of P. borrori. Nematodesmal microtubules (Ne) anastosmose with microtubules of the posterior cytoskeleton (Cy). Single postciliary microtubules (arrows) are aossociated with internal cirral kinetosomes. (X 11 800)


Dzm
Db
Dc
Plm
Cc
39

in P. borrori; this process, however, enabled me to identify the general developmental pattern (similar to that of D. ehrenbergi) and to trace the source of various cirral organelles.

Frontal ciliature develops from 9 streaks. These streaks divide into 4 transverse ranks of procirri. The first (posteriormost) forms the transverse cirri, the second forms 2 accessory transverse and from the third and fourth ranks develop the remaining frontal cirri, including midfrontal, malar, and from the rightmost procirri, 2 migratory cirri. A paroral cirrus appears to develop from the dedifferentiated parental paroral apparatus. Right and left marginal cirri arise by within row development. Two groups of cirri differentiate from the left marginal primordium: the anterior cirral row and the posterolateral group. Caudal cirri develop from the right most dorsal kineties.

Psammocephalus dragescoi n. sp.

I discovered P. dragescoi n. sp. (fig. 41) in medium-fine sand between the mean tidal level and mean low water of Foss Beach, New Hampshire (type locality: 43°0'24" lat., 70°44'30" long.). It appears to be associated with sediments between 3-6 cm in depth. This species is named in honor of Dr. Jean Dragesco.

Morphology

This thigmotactic ciliate (approximately 85 μm in length) is cephalized with a flexible peristomial lobe anteriorly and is fairly supple posteriorly. The ciliature of the cephalized region includes 3 frontal cirri, a paroral cirrus, 5-6 collar membranelles, approximately 12 lapel membranelles, and an endoral and paroral membrane. The anterior end of the paroral membrane is differentiated into a peristomial cirrus. Posteriorly the cell possesses right and left marginal

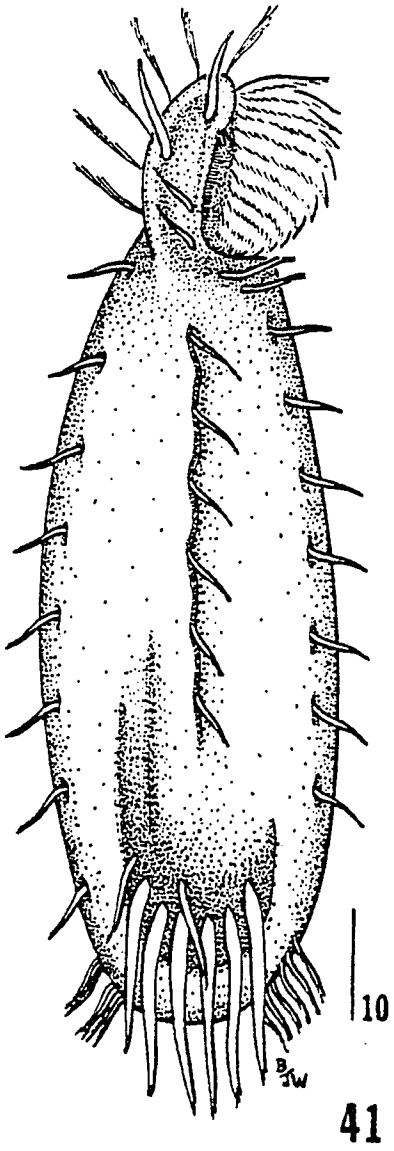


Fig. 41. Ink drawing of Psammocephalus dragescoi n.gen., n.sp.

cirri, a longitudinal series of midfrontal cirri, 2 accessory transverse cirri, and a U-shaped group of transverse cirri that emerge from a ventral concavity. The posterior end of the left marginal cirral row is differentiated into a set of 4-6 posterolateral cirri. On the dorsal surface are six bristle rows with long (7 μm) bristle cilia; 2 or 3 groups of caudal cirri are associated with the rightmost dorsal kineties.

The 12-20 macronuclei are arranged in 2 longitudinal arrays on either side of the midfrontal cirri. A postbuccal inclusion (7 μm in diameter) containing both a crystalline and an opaque particle is present in P. dragescoi. Food vacuoles contain various kinds of diatoms.

Psammocephalus faurei (DRAGESCO, 1963) n.comb.

P. faurei was first described by DRAGESCO, 1963, from a population in fine marine sand at Roscoff, France. I discovered an additional population of this species (fig. 42) in sand near mean low water of Plum Island, Mass.; the individuals of this population range from 80 to 175 μm in length and are supple with a flexible cephalized anterior. This species appears less thigmotactic than D. ehrenbergi.

Morphology

The cephalized region forms a ventral peristomial lobe; the ciliature of this region consists of 3 frontal cirri, a paroral cirrus, 9 collar membranelles, approximately 25 lapel membranelles and an endoral and paroral membrane. The anterior end of the paroral membrane is differentiated into a peristomial cirrus. On the posterior portion of the cell there are right and left rows of marginal cirri; the

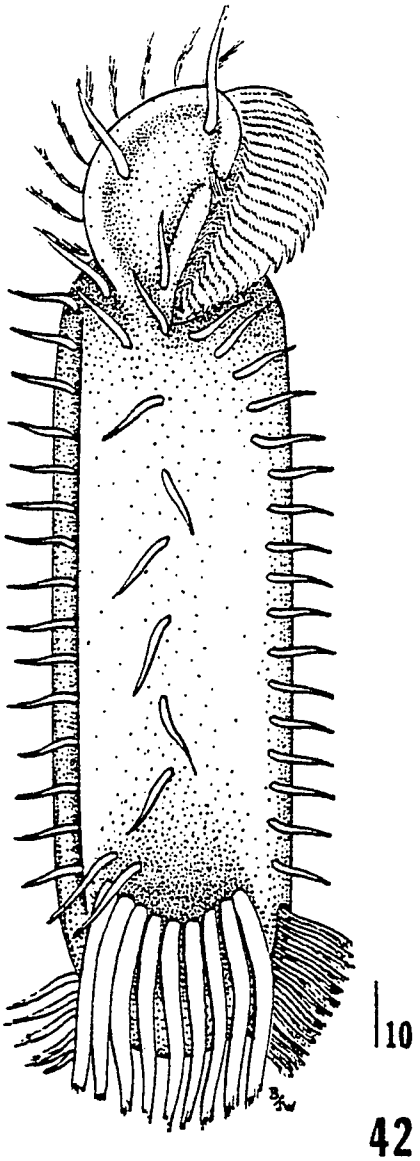


Fig. 42. Ink drawing of Psammocephalus faurei (DRAGESCO, 1963) n.comb.

left marginal cirri are divided into a row of 17-19 anterior cirri and a posterolateral group of approximately 14 cirri. A longitudinal series of midfrontal cirri, subtended by a U-shaped group of 8-10 transverse cirri and 2 accessory transverse cirri, are also present, as well as 2 migratory cirri (located just posterior to the distal collar membranelles). Although the cytoskeleton is less complex than D. ehrenbergi, a posterior cytoskeletal trunk, formed by the union of large anterior microtubular bundles of each transverse cirrus, is present.

On the dorsal surface are 6 rows of bristles; most rows consists of approximately 15 bristle complexes, each bearing a long (6-7 μm) cilium. Row 4, however, consists entirely of short (3 μm) bristles. Although most of the bristle complexes of rows 2 and 3 possess long cilia, the anterior ends of both these rows (on the cephalized portion of the cell) possess short (3 μm) cilia. Caudal cirri are associated with the right most dorsal kineties. Numerous kinds of diatoms are found within the food vacuoles. The cell is multimacronucleate possessing 16-35 macronuclei.

Morphogenesis

I observed both morphogenesis of re-organization and an incomplete series of morphogenesis during cell division in P. faurei. During re-organization a longitudinal series of 10-12 oblique streaks are formed on the ventral surface: from the leftmost streak differentiates the paroral apparatus including the paroral cirrus; from the remaining streaks differentiate 4 oblique (later transverse) ranks of frontal cirri. The first (posteriormost) rank forms the transverse cirri; the second rank forms the accessory transverse cirri only; the third

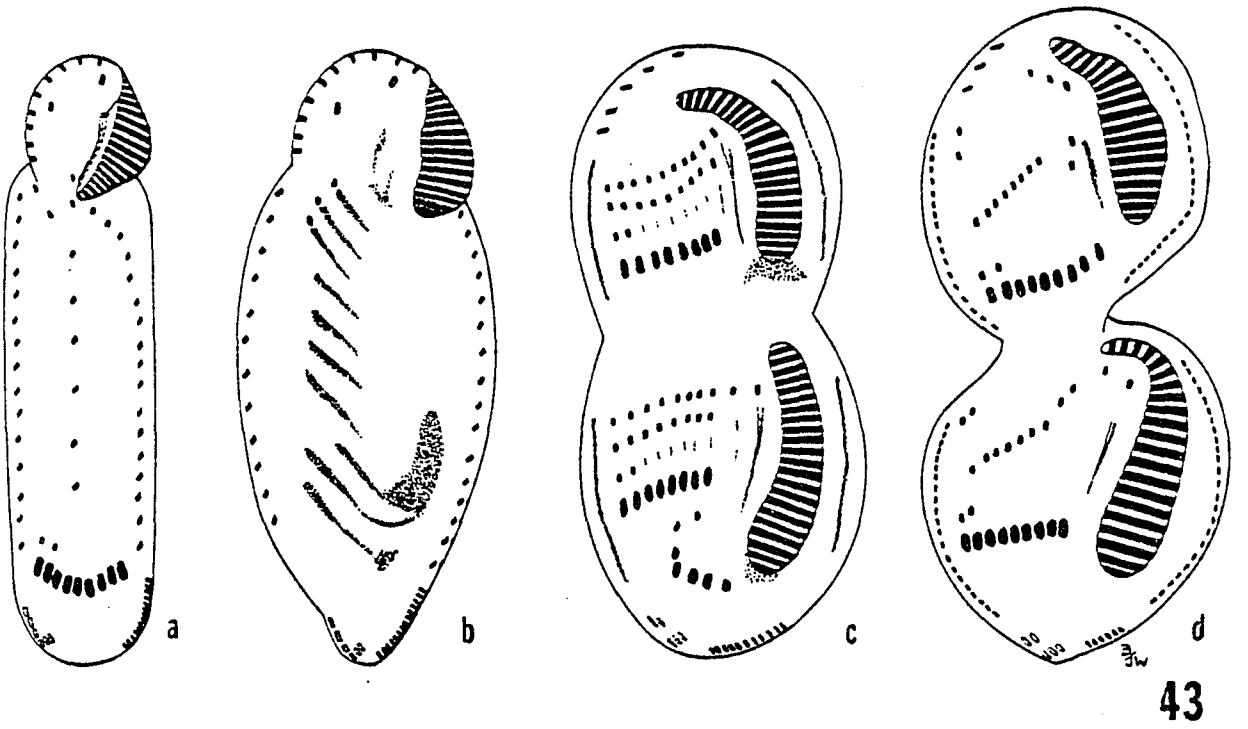


Fig. 43. Line diagrams of a non-dividing cell (fig. 43a) and a sequence of ventral, morphogenetic stages during cell division (fig. 43b,c,d) in P. faurei. Black areas represent ciliary organelles.

rank forms the longitudinal series of midfrontal cirri and, from rightmost procirrus, a migratory cirrus; the fourth rank forms the second migratory cirrus and the remaining anterior frontal cirri. The migratory cirri are later positioned just posterior to the distal collar membranelles. Right and left marginal cirri arise by within row development. The left marginal streak divides to form the anterior marginal row and the posterolateral marginal group. Caudal cirri develop from the rightmost dorsal kineties. Parental membranelles dedifferentiate (at least partially), then reform.

A similar process occurs during cell division morphogenesis (figs. 43a-d). First, an oral primordium and a longitudinal series of oblique streaks arise on the cell surface while the parental paroral apparatus and midfrontal cirri dedifferentiate. Two frontal fields, each consisting of 4 transverse ranks of procirri, appear by mid-division - each rank develops a similar manner as during re-organization (fig. 42c). More procirri develop from frontal streaks than are present in late division (when they are presumed to be resorbed). The paroral apparatus, paroral cirrus, migratory cirri, marginal cirri and caudal cirri all differentiate as in re-organization morphogenesis (fig. 42c,d).

Psammocephalus lithophora (FAURÉ-FREMIET, 1954) n.comb.

In 1954 FAURÉ-FREMIET described the marine interstitial hypotrich P. lithophora (fig. 44) from surface sands (0.15-0.40 μm) of the Concarneau region of France. This is an elongate (120-135 μm) strongly thigmotactic ciliate with a marked cephalization: the anterior cephalized region is supple and mobile while the posterior portion of the cell is rigid. Right and left marginal cirri and a longitudinal series of frontal cirri emerge from cortical grooves on the ventral surface.

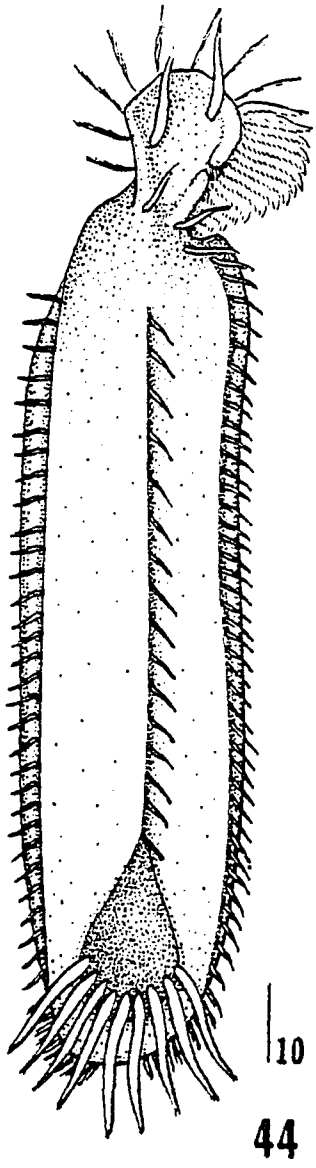


Fig. 44. Ink drawing of Psammocephalus lithophora (FAURÉ-FREMIET, 1954) n.comb. (after FAURÉ-FREMIET, 1954).

A U-shaped group of 7-9 transverse cirri lie within a ventral concavity that is continuous with the midventral groove. A peristomial lip borders the cephalized region ventrally; from a cleft in this lip, just right of the lapel membranelles, extend a small group of cilia that may represent an anterior differentiation of the paroral membrane - presumably a peristomial cirrus: "un sillon olbique occupé par une courte rangée de cirres fins que répresentent, vraisemblablement, les cils parorau". Just below the cepalized region, on the right side of the cell, is a statocyst-like inclusion - the "vacuole à concrétion" (FAURÉ-FREMIET, 1954).

Amphisiella marioni GOURRET and ROESER, 1887

Morphology

A. marioni is a marine benthic hypotrich (75-125 µm in length), that is supple and slightly contractile. The ventral ciliature comprises right and left marginal cirri, a paroral cirrus, approximately 7 sporadically positioned anterior frontal cirri, 4-5 transverse cirri, 2 accessory transverse cirri, a longitudinal row of about 22 median cirri, and although not described by previous observers (probably because of its close proximity to the anterior of the median row), an additional short (4-7 cirri), frontal row lying just posterior to the distal membranelles (fig. 45a). The buccal ciliature includes approximately 13 collar and 22 lapel membranelles, and a paroral and endoral membrane; there is no differentiation at the anterior end of the paroral membrane. Six dorsal kineties are present, caudal cirri are absent. Only 2 macronuclei are present.

Morphogenesis

Cell division morphogenesis in A. marioni begins with the prolifera-

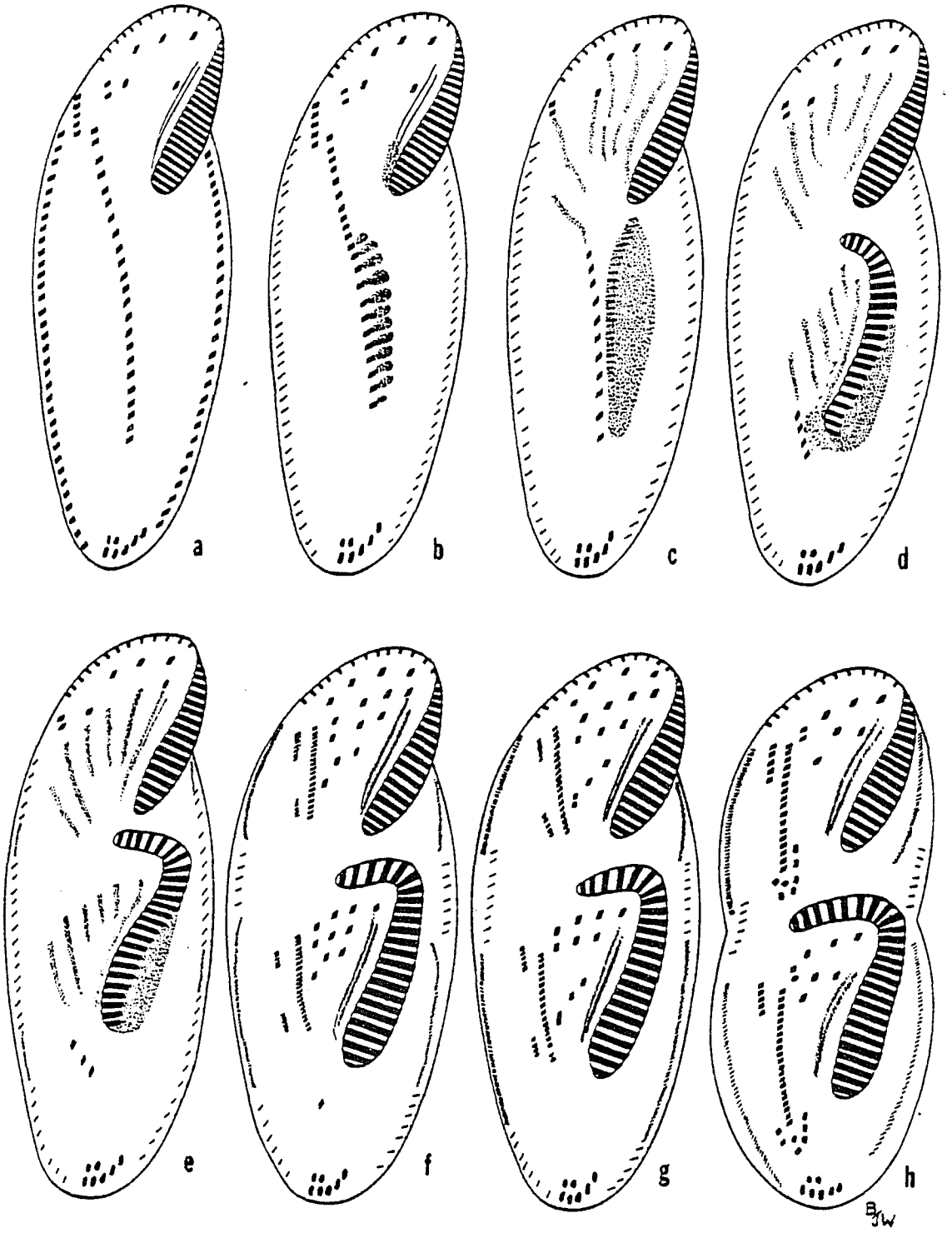


Fig. 45. Line diagrams of a non-dividing cell (fig. 45a) and a sequence of ventral, morphogenetic stages during cell division (fig. 45b-h) of Amphisiella marioni.

tion of kinetosomes beside each of the 10-12 median row cirri; these form a series of primordia (fig. 45b) that enlarge into an extensive kinetosomal field (fig. 45c). Five frontal streaks and the paroral streak, as well as the oral primordium, arise from this opisthe field (fig. 45d). Meanwhile, the parental paroral apparatus dedifferentiates while 5 frontal streaks form in close association with 3 anterior frontal cirri, the anteriormost median cirri and the short row of post-collar-membranelle cirri.

As development proceeds in the opisthe, membranelles are formed and the endoral and paroral membranes differentiate from the paroral streak; a paroral cirrus develops from the anterior end of the paroral membrane. Three procirri develop from each of the 3 leftmost frontal streaks: the posterior member of each of these rows of procirri migrates posteriorly to form transverse cirri; the remaining 6 procirri form anterior frontal cirri, including a malar cirrus (fig. 45e,f,g). Most of the fourth frontal streak differentiates median row cirri; the posteriormost procirrus of the row, however, forms an accessory transverse cirrus. The fifth (rightmost) streak splits into 2 segments during development - the anterior segment forms the short right lateral row, the posterior segment contributes a transverse and an accessory transverse cirrus (fig. 45g,h). Dorsal kineties and marginal cirral rows arise by within row development.

Discussion

Geographic Variation of D. ehrenbergi

Discocephalus ehrenbergi is a widely distributed interstitial ciliate (HARTWIG and PARKER, 1977). In populations from the coast of Europe and both east and west coasts of North America (table 1), the numbers of dorsal kineties and anterior marginal cirri appear stable whereas the numbers of transverse cirri, sets of caudal cirri, and cirri within caudal sets are variable. Although sample size is low, individuals from all locations evidence a similar morphology and cirral pattern.

Because encystment and planktonic larval stages are absent in D. ehrenbergi (as in many other marine interstitial species), its means of dispersal remains enigmatic. STERRER (1973) and CORLISS and HARTWIG (1977) suggested movement of tectonic plates as a method of dispersal of interstitial fauna. The fragmentation of the supercontinent Pangea during the Jurassic and Cretaceous periods could account for the distribution pattern of D. ehrenbergi. Other passive modes of dispersal, however, are also plausible. Rafting of sediment on drifting objects, although considered rare, may be persistent through time (GERLAC, 1977). Indirect evidence exists for aerial transport by birds; due to the absence of dessication resistant cysts or eggs, however, dispersal by this means is considered minimal (FENCHEL, 1978). Ciliate biogeography is further complicated by the existence of morphologically inseparable species complexes (BORROR, 1980). The autecology and population dynamics of organisms, evolutionary

mechanisms and environmental conditions are all involved in protist biogeography (BAMFORTH, 1981). If, however, distribution patterns of interstitial fauna have occurred through continental drift processes, an indirect method of estimating minimal geological age of a species may be made possible.

Discocephalus-like Hypotrichs as an Evolutionary Assemblage

Discocephalus-like hypotrichs, D. ehrenbergi, D. rotatorius, P. minimus, P. borrori, P. dragescoi, M. grandis, P. faurei, and P.lithophora, represent a unique and diverse, yet highly cohesive group of ciliates. Diversity within the group is apparent in the great variety of cell shapes, the wide range of both cirral number and hypertrophy, and the varied degree of cytoskeletal complexity among the different species. The cohesiveness is due to a series of ecological, behavioral, morphological and morphogenetic characters.

At least 3 species, D. ehrenbergi, P. borrori, and P. dragescoi, co-occur at Foss Beach. Fine, well sorted sand (SCOTT and CROKER, 1976) and high interstitial water content characterize this moderately exposed beach. Sediments, however, can change drastically during natural catastrophies: during the winter of 1976-1977, a storm reduced portions of the intertidal zone to large rocks and peat. Niche divergence by means of differences in vertical distribution patterns or in food size selection (as shown for 3 species of Remanella by FENCHEL, 1968) may explain the coexistence of the above species. Each species exhibits similar behavioral adaptations, such as erratic back and forth movements when feeding and rapid reverse spiralling movements when disturbed. In addition, the above species occur intertidally during the cooler seasons from September to December and from April to June; they were

not found either in midwinter or midsummer. I presume they migrate subtidally.

The most obvious shared morphological feature is cephalization: all species have a strongly cephalized, mobile anterior region that forms a ventral peristomial lobe and houses the buccal apparatus and 2 prominent anterior cirri (the paroral cirrus and the anteriormost streak II cirrus). From a cleft in the left side of the peristomial lobe protrudes a unique differentiation of the paroral membrane - the peristomial cirrus. The posterior region of the cell bears a midfrontal cirral row (reduced in some species), a short right frontal row, a set of transverse cirri disposed in a U-shaped group, migratory cirri, and left marginal cirri which differentiate into 2 distinct sets. Dorsal cilia are long (7 µm) and caudal cirri are arranged in sets derived from different dorsal kineties. The microtubular cytoskeleton is divided into 2 major units: an anterior component of the cephalized region and a posterior component dominated by a microtubular trunk formed by the union of transverse cirral microtubular bundles. The cytoskeleton reaches its greatest complexity in D. ehrenbergi.

Certain features of discocephalids are also present in other hypotrichs. For example, cephalization is an adaptive specialization shared to greater or lesser degrees, by such hypotrichs as Epiclintes ambiguus (MÜLLER, 1786), Gastrostyla stenocephala (BORROR, 1963), and Trachelostyla pediculiformis (COHN, 1866). Positional and compositional criteria can be used to determine homology: structures or functions are homologous when they occupy similar positions and are composed of similar components in organisms of which they are part (see HANSON,

1976). A comparison of compositional and positional relationships of cirral structures, membranelles, and paroral apparatuses, as well as the pattern of development of these structures between the above hypotrichs and discocephalids indicates non-homologous forms of cephalization. Hence, I consider the cephalization in these species as convergent with that of discocephalids. Within the discocephalids, however, it is the minuteness of resemblance in position and composition of structures of the cephalized region (e.g. the peristomial lobe, peristomial cirrus, buccal cleft, cytoskeletal organization) that demonstrates homology.

During morphogenesis (discussed below) in each species studied, the arrangement of cirral anlagen, deployment of procirri and their subsequent cortical positioning demonstrate a homologous developmental pattern unique to the discocephalids.

Comparative Ultrastructure

Hypotrich cirri, membranelles, and dorsal bristles can be considered organellar complexes: each possesses a fundamental structure that is homologous throughout the order and in some cases throughout the entire subclass and beyond (PUYTORAC, 1976; WICKLOW, 1981). Although some quantitative structural variation is present, this level of organization is considered moderately conservative (LYNN, 1976). In hypotrichs for example, the arrangement and direction of frontal cirral microtubular bundles differ between urostylid and oxytrichid species (WICKLOW, 1981a). In oxytrichids, anterior frontal cirri are linked with collar membranelles; this linkage is absent in urostylids, but right and left midventral (frontal) cirri may be linked in a latter-like array. Modifications about a common structural theme

may indicate recent evolutionary divergence and help trace phylogentic pathways.

Cirri. In Discocephalus ehrenbergi the position of microtubular ribbons in relation to both the cirrus and the general cell orientation is similar to that in the hypotrichs Gastrostyla (GRIM, 1972), Oxytricha (GRIMES, 1972), Paraurostyla (GRIMES and L'HERNAULT, 1978, JERKA-DZIADOSZ, 1980), Stylonychia (PUYTORAC et. al., 1976) and Thigmokeronopsis (WICKLOW, 1981a) as well as the heterotrich Plagiotoma (ALBARET and GRAIN, 1973). Whereas the number of microtubules composing each ribbon may vary within and between species, the ribbon position is conservative, reflecting developmental homologies.

The pattern of kinetosomal connectives within cirri of D. ehrenbergi, however, differs from the above-mentioned genera, but appears more similar to the connectives described in Euplotes (TUFFRAU et. al., 1968): connectives anastomize between cirral kinetosomes in both species, forming a similar reticulate pattern that is repeated throughout the cirral base. Microtubular bundles in cirri of D. ehrenbergi - particularly those associated with the transverse cirri - provide a unifying feature to the family while sharing little similarity with other hypotrichs.

Buccal apparatus. Hypotrichs, as well as heterotrichine heterotrichs, share a similar membranellar structure in the form of paramembranelles (PUYTORAC and GRAIN, 1976). The hypotrich paroral apparatus is more variable: while the endoral membrane remains as a single kinetosomal row in all genera, the paroral membrane can range among species from a single kinetosomal row (together with the endoral membrane termed diplostichomonade) to a paroral membrane comprising

multiple kinetosomal arrays (polystichomonade). This quantitative difference varies both within and between hypotrich families. JERKA-DZIADOSZ (1981b), by examining the ultrastructural morphogenesis of the paroral apparatus, demonstrated that the polystichomonad paroral membrane of Paraurostyla weissei develops by the addition of kinetosomes to each rightmost member of a longitudinal series of kinetosomal pairs after the leftmost kinetosome of each pair is split off to form the endoral membrane. Different numbers of kinetosomes added during this late developmental phase can account for the variation.

The differentiation of a peristomial cirrus in discocephalids represents a significant divergence in paroral apparatus formation in hypotrichs. All hypotrichs have at least one paroral cirrus derived from the paroral membrane during late development; these cirri are structurally and functionally similar to locomotory frontal cirri. The peristomial cirrus, as a linear array of kinetosomal doublets surrounded by an electron dense fibrillar matrix, however, appears intermediate between membrane and cirrus and functions as part of the buccal apparatus.

Dorsal bristle complex. The kinetosomal pair represents a fundamental structural unit from which more involved organellar complexes, such as cirri and membranelles, develop (GRIMES, 1972; JERKA-DZIADOSZ, 1981a; RUFFOLO, 1976). Dorsal bristles are the simplest form of the hypotrich kinetosomal complex; they consist of a kinetosomal pair with the anterior kinetosome ciliated, the posterior kinetosome nonciliated, and may include such accessory microtubular and fibrillar derivatives as postciliary, transverse, and nematodesmal microtubules, fibrous connectives (desmoses), and a kinetodesmal fiber. Due to

the likely conservative nature of this level of organization, somatic kinetid ultrastructure has been proposed as a powerful tool in tracing ciliate phylogeny (GIERASSIMOVA and SERAVIN, 1976; LYNN, 1976). Ultrastructural studies have revealed both intriguing similarities as well as significant differences in kinetid organization between ciliate groups. The somatic kinetosomal pairs (dikenetids) of karyorelectid gymnostome cilates and heterotrich cilates bear postciliary microtubular ribbons that overlap in a series as a postciliodesma. Hypotrichs also possess overlapping postciliary microtubules in their somatic kineties (e.g. see fig. 29 in GRIMES and ADLER, 1976) and in their buccal kineties within membranelles (WICKLOW, 1981a, see also fig. 15). Colpodids possess an overlapping series of posterior kinetosomal transverse microtubules within the kinety - the LKm fiber (GOLDER and LYNN, 1980). The colpodid Bursaria truncatella, however, while possessing an LKm fiber, also has an overlapping (but shorter) series of postciliary microtubules (LYNN, 1980; PEREZ-PANIAGUA et. al., 1980). This dual nature of the overlapping microtubules of Bursaria, as well as similarities in position of other somatic microtubular derivatives and interkinetosomal connectives with spirotrichs such as Discocephalus, support an intriguing supposition: the colpodids and spirotrichs may have diverged from the same evolutionary line - the colpodids exploiting the LKm fiber system, while the spirotrichs continued to exploit a system based on the postciliodesma. Furthermore, similarities exist between colpodid cirromembranelles and spirotrich paramembranelles that may also prove to be homologous (WICKLOW, 1981a). SERAVIN and GIERASSIMOVA (1978) proposed a lineage between karyorelictid and spirotrich ciliates that bypasses the Oligohymemophora. GOLDER

and LYNN (1980), although suggesting the colpodid LKm system represents, in addition to the postciliodesma and kinetodesma, a third major category of somatic, kinetid organization, also proposed a possible common ancestry between colpodids, karyorelictids, and spirotrichs. More studies of ultrastructural morphogenesis may help elucidate these relationships.

When comparing dorsal bristle structure of D. ehrenbergi with that of other hypotrichs, both similarities and subtle, yet in some cases significant, differerences are revealed. As in D. ehrenbergi (and colpodids such as Bursaria), both bristle kinetosomes of Oxytricha (GRIMES, and ADLER, 1976) bear posticilary microtubules: a single microtubule associated with the anterior kinetosome and 3 microtubules associated with the posterior kinetosome. Postciliary microtubules are apparently absent in the anterior bristle kinetosome in Euplotes (RUFFOLO, 1976). A fibrillar mass to the left of the bristle pairs in Oxytricha is also present in D. ehrenbergi but lacking in Euplotes. A kinetodesmal fiber is associated with the posterior kinetosome of mature bristle complexes of Discocephalus, Euplotes, and Certesia, but is present only in the immature bristle complex of Oxytricha. RUFFOLO (1976) described a network of particles ("lasiosomes") within the bristle cilium of Euplotes; these particles are also present in the bristle cilium of Certesia (WICKLOW, unpubl.) but absent in Discocephalus.

A unique feature of the bristle complex in Discocephalus is the basket-like framework that surrounds the bristle pair. Lacking homologues in bristle complexes of other hypotrichs, this framework appears similar to the peripheral fibrillar matrix of hypotrich cirri.

The general organization of the bristle complex agrees with the hypotrich dikinetid pattern characterized by LYNN (1981); the fibrillar framework however suggests a large diversity of ancillary fibrillar structures associated with hypotrich dikinetids.

Morphogenesis

Just as cell structures can be understood in terms of increasing levels of organization with more complex levels based on the stability of preceeding levels (hence the Structural Conservatism Hypothesis of LYNN, 1976), so too can morphogenetic processes be viewed as a series of sequential events: each succeeding step is based on satisfactory completion of a preceeding event. As an extension of LYNN's hypothesis, primary developmental events would be highly conservative; variability increases as more complex differentiation occurs. Dissimilarities at the organellar or organellar complex level of development would indicate early ancestral divergence while similarities in development at this level are expected between ciliates of a common evolutionary lineage.

JERKA-DZIADOSZ (1981a), in an ultrastructural morphogenetic study of Paraurostyla weissei, followed the events leading to membranelle formation. During this process a double-row promembranelle is formed by the alignment (proceeding from the cell's right to left) of kinetosomal pairs, each with postciliary microtubules directed posteriorly. These pairs are derived from an anarchic field of randomly distributed pairs. Homologous processes also appear to operate in membranelle formation in Oxytricha (GRIMES, 1972) and Euplotes (RUFFOLO, 1976). Paramembranellar morphogenesis is predicted to proceed in the same manner in all hypotrichs, heterotrichine heterotrichs, and perhaps

other groups.

Developmental changes that have occurred during cilate evolution may be related to the timing of morphogenetic events. For example, cirromembranelles in the vestibuliferan Bursaria originate from somatic kineties; each kinety provides at least the initial kinetosomes for one membranelle (PEREZ-PANIAGUA et. al., 1980). Cirral rows (modified somatic kineties) are also directly involved in stomatogenesis in other groups of ciliates such as the Polyhymenophora: heterotrichs, Climacostamum (DUBOCHET et al., 1979), Plagiotoma (ALBARET, 1973), and hypotrichs, Kahliella (TUFFRAU, 1969), Pseudourostyla (JERKA-DZIADOSZ, 1972). Although stomatogenesis in both Bursaria and polyhymenophorans is based on the kinetosomal doublet as a formative unit (with 1 or 2 additional kinetosomes added to promembranelles as development proceeds) the additional period of development of an oral primordium is present in the Polyhymenophora. The formation of an oral primordium is presumed to have accompanied concomitantly the reduction of somatic kineties (oligomerization) during ciliate evolution.

Once a primordium is formed, deployment of incipient organelles or organellar complexes such as hypotrich cirri results in various morphogenetic patterns (higher level development events) that can be used to identify homologous structures and demonstrate recent ancestry and which are described in later paragraphs. Several distinct developmental patterns have been reported in hypotrichs: the euplotine pattern, e.g. Certesia (WICKLOW, 1979), Diophrys (HILL, 1981), Euplotes (RUFFOLO, 1976), the sporadotrichine pattern, e.g. Gastrostyla (WALKER and GRIM, 1972), Oxytricha (GRIMES, 1972), Paraurostyla (GRIMES and

L'HERNAULT, 1978), the stichotrichine pattern, e.g. Cladotricha (BORROR, 1979), Hypotrichidium (TUFFRAU, 1972), Kahliella (TUFFRAU, 1969), and the urostyline pattern, e.g. Holosticha (HILL, 1980), Thigmokeronopsis (WICKLOW, 1981a), Urostyla (BORROR, 1979). Cortical morphogenesis in Discocephalus demonstrates an additional developmental pattern, divergent from other hypotrichs.

In most hypotrichs development proceeds in 2 latitudinal developmental zones: an anterior zone of the future proter, and a posterior zone of the future opisthe, although an exception appears in the sporadotrichine Tachysoma (CULBERSON, 1979). Most euplotine hypotrichs exhibit only 1 latitudinal proliferative zone that later splits into proter and opisthe developmental fields. In Euplotes frontal ciliature develops as a double set (apparently de novo), while dorsal bristles develop as a single set. I consider development in Euplotes as occurring in 1 determinative region, and hence, a modified single zone type. Cell division morphogenesis in Discocephalus also occurs in a single proliferative zone.

The new buccal apparatus in euplotines arises within a subcortical pouch; in Discocephalus, as in most other hypotrichs, the new buccal apparatus develops from an oral primordium at the cell surface.

Euplotines have separate buccal and frontal primodia; the frontal streaks in Discocephalus develop as a lace-like network contiguous with the oral primordium. These frontal anlagen later form a series of up to 10 oblique streaks similar to the midfrontal streaks of urostylines. Each frontal streak in discocephalids differentiates into a series of procirri arranged in 4 ranks in a similar way as occurs in urostylines such as Keronopsis (WICKLOW, 1981a). The first

(posteriormost) rank forms the transverse cirri; the second rank forms the accessory transverse cirri in both discocephalids and in the posterior frontal field of Keronopsis; the third and fourth rank in Keronopsis form the double series of midventral cirri characteristic of the Urostylina, whereas, in discocephalids a single median series of midfrontal cirri is formed from the third procirral rank while the fourth procirral rank contributes the right frontal cirri. Although the right frontal cirri are reduced to a short series, appearing divergent in function and position from the right midventral row in urostylines, I believe the right frontal row and the midfrontal row in discecephalids to be homologous to the midventral ciliature of urostylines. In addition, the right anterolateral - migratory - cirrus of discocephalids is so similar in origin and later positioning to the migratory cirri of urostylines (WICKLOW, 1981a) as to also be homologous.

The midfrontal ciliature composing the median row in discocephalids is, however, non-homologous to the median row of other hypotrichs (BORROR and WICKLOW, 1981). An example of this non-homology is the median row in Amphisiella marioni: each cirrus within the row originates within the same frontal streak, whereas each median row cirrus in discocephalids originates from different frontal streaks. The morphogenetic pattern of A. marioni is homologuous to that of Gastrostyla indicating a close phylogenetic relationship with sporadotrichine hypotrichs (WICKLOW, 1981b).

The morphogenetic pattern of Psammocephalus faurei n.comb. (formerly placed in the genus Amphisiella) contrasts the pattern of A. marioni and is homologous to the Discocephalus pattern. Hence the classifica-

tion of P. faurei in the genus Amphisiella is artificial; both morphological and morphogenetic data demonstrate P. faurei to be in the discocephalid lineage.

Development of somatic ciliature in discocephalids - marginal cirri, caudal cirri, and dorsal bristles - bear similarities with euplotine hypotrichs. Dorsal bristle primordia split from 1 to 2 fields in both groups; caudal cirri then develop from the rightmost dorsal kineties. Right marginal cirri are present in many discocephalids, but are absent in D. ehrenbergi and the euplotines. Left marginal cirri arise from a single within-row primordia in D. ehrenbergi, then form an anterior marginal set in a similar way as in Certesia. The differentiation of a separate group of posterolateral marginal cirri in discocephalids, however, is unique with no homologue in other hypotrichs studied (a possible exception is Erionella discussed below).

Although homologies may exist, cortical morphogenesis in Discocephalus represents a divergent developmental pattern that unites a species group ranging from members with elongate cell shapes and long rows of midfrontal cirri, to those with oval cell shapes and reduced midfrontal rows. This pattern, along with other features discussed above, is sufficiently divergent to warrant separation of Discocephalus-like hypotrichs into their own suborder: the Discocephalina.

Adaptive Radiation

Discocephaline adaptive radiation is reflected in cell shape, number and size of cirri and cirral rows, and the degree of cephalization and cytoskeleton complexity. Divergence within this geneology is

presumed to be related to the heterogeneity of marine sands. This includes differences in horizontal and vertical parameters such as grain size and microporal water content as well as adaptation to different microfloral food sources.

The adaptive significance of cephalization appears to be related to feeding strategies: a cephalized, anterior feeding apparatus allows utilization of a wider range of feeding surfaces; the ability to flex this cephalized region (and thereby conforming to the feeding surface) further enhances feeding efficiency.

Another striking feature of Discocephalus is the extensive microtubular cytoskeleton. Microtubular systems are found in all eukaryotic cells. General function of these systems include chromosomal movement during mitosis, intracellular movement and communication, maintenance of cell form and integrity, and ciliary motion. Microtubular systems are considered to have reached their most complex state in ciliates (LYNN, 1981); this complexity has culminated in the microtubular system of Discocephalus.

The microtubular cytoskeleton of Discocephalus ehrenbergi is in two parts - an anterior system within the cephalized region and a posterior system. This discontinuity allows articulation of the cephalized region of the cell. Several additional functions may be attributed to the microtubular system in Discocephalus: cirral anchorage, maintenance of cell form and integrity, and in the form of the dorsal antler and cortical spines as possible protective or sensory mechanisms.

The extensive cytoskeleton in Discocephalus is an adaptive specialization to interstitial life on exposed beaches. Microtubules

furnish both endoskeletal as well as subcortical components as a means to insure maintenance of structural integrity and cell form of non-contractile ciliates in a turbulent environment. Supplemental methods of structural maintenance have been observed in psammophilic ciliates with less developed microtubular cytoskeletals. For example, Discotricha papillifera has an epiplasma-like layer below its plasma membrane (WICKLOW and BORROR, 1977). Euplotes vannus possesses proteinacious, alveolar plates which may contribute to cell rigidity (HAUSMANN and KAISER, 1979; BÖHM and HAUSMANN, 1981). Certesia quadrinucleata also possesses alveolar plates in addition to cortical microtubules. D. ehrenbergi lacks both an epiplasma layer and alveolar plates - cell form is apparently maintained by microtubules alone.

Entodiniomorphs such as Ophryoscolex and the trichostome Isotricha have cytoskeletal features that parallel Discocephalus. Orphyroscolex candatus possesses an extensive fibrillar endoskeleton; Isotricha prostoma also has a fibrillar network that supports both its cytostome and nuclei. Both these ciliates are endosymbionts within the rumen of herbivores - perhaps representing a kind of interstitial habitat.

Microtubular systems provide Discocephalus a structural adaptive strategy for interstitial life. The value of microtubules in this strategy lies in their high degree of functional versatility, capability of being rapidly dismantled or resorbed and their potential for rapid assembly during morphogenesis; they are non-restrictive to cell growth and provide structural integrity without severe encumbrance.

Phylogeny

Somatic ciliature (i.e. ciliature developing by within row kinetosome proliferation) is present in the form of kineties comprising

kinetosomal pairs in most heterotrichs such as Condylostoma and Climacostomum or in meridional rows of cirri (modified kineties) in some hypotrichs and advanced heterotrichs such as Plagiotoma and Transitella. Hypotrich somatic meridional rows may be distributed dorsally and ventrally, as in Kahliella, or limited, as varying numbers of marginal cirral rows, to the ventral surface as in most other hypotrichs. Somatic ciliature is a generally shared trait in hypotrichs; hence, it is evolutionarily conservative and considered an ancestral trait or plesioseme. Frontal ciliature, a newly evolved trait particular to hypotrichs, is considered an aposome.

DOGIEL (1929) and later POLJANSKI and RAIKOV (1976), proposed the hypothesis that the process of polymerization and oligomerization accompanied ciliate evolution: an increase of ciliary organelles, polymerization, is associated with early ciliate evolution; a decrease in ciliary organelles, oligomerization (by reduction, fusion, or change in function) is associated with more recent ciliate evolution. Kahliella, with many somatic meridional rows and limited frontal ciliature, is generally considered as among the most "primitive" of hypotrichs (BORROR, 1979a, CORLISS, 1979a, TUFFRAU, 1979). Once the hypotrich grade was reached, further evolution is presumed to proceed by oligomerization of both cirral rows and cirral number, and by decreased emphasis on somatic ciliature with increased exploitation of frontal ciliature.

Marked differences in number and degree of hypertrophy in both somatic and frontal cirri occur between members of the Discocephalina. These differences in ciliature are associated with differences in cell shape and degree of cytoskeletal complexity. For example, P. faurei

has complete rows of right and left marginal cirri as well as a complete midfrontal series; it is extremely elongate with a low degree of cytoskeletal complexity. P. borrori has complete left and right marginal cirral rows but a reduced midfrontal series; it is less elongate with a moderate degree of cytoskeletal complexity. D. ehrenbergi has an extremely reduced but hypertropied left marginal row and midfrontal series, while right marginal cirri are completely absent; it is oval with a high degree of cytoskeletal complexity.

This series of changes demonstrates an evolutionary trend in discocephalines toward increased cytoskeletal complexity, cirral reduction and hypertrophy, and ovoid cell shape. In this evolutionary series, P. borrori and P. dragescoi serve as intermediate forms linking advanced species such as D. ehrenbergi with more "primitive" species such as P. lithophora (fig. 46).

Up to this point I have been concerned mainly with what makes up a discocephaline - those characters that unify the group as a distinct evolutionary assemblage - and how evolution within the group may have proceeded. The questions of from what group the discocephalines may have stemmed and what group(s), if any, diverged from the discocephaline lineage remain to be discussed.

The "primitive" discocephalines, such as P. faurei and P. lithophora, bear no resemblance to members of the genus Amphisiella. For example, the median rows of the 2 groups are non-homologous. Frontal ciliature of discocephalines, however, appears homologous (although modified) to the frontal ciliature of urostylines. Furthermore, since all discocephalines are multimacronucleate, their divergence from a multimacronucleate ancestor is likely. The multimacronuclearity

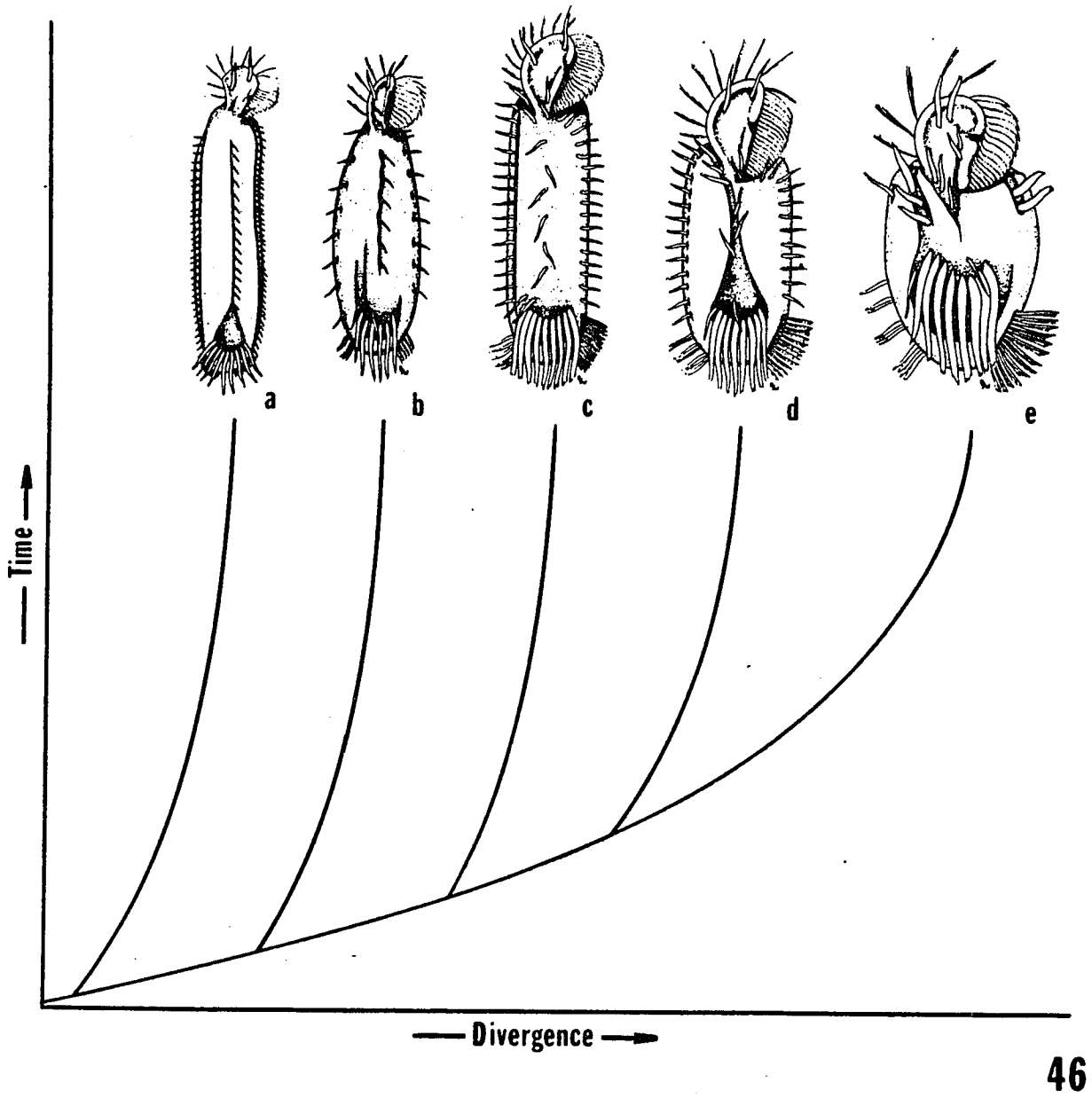


Fig. 46. A proposed evolutionary series of discocephaline hypotrichs. a. P. lithophora; b. P. dragescoi; c. P. faurei; d. P. borrori; e. D. ehrenbergi.

of many marine urostylines satisfies this condition.

Cephalization is prominent in the urostylines Holosticha lacazei and Holosticha alveolata, but is most striking in the multimacronucleate, marine interstitial, Holosticha discocephalus. BORROR, in his unpublished observations of H. discocephalus from sands of Alligator Harbor, Florida, describes the species as having 12 pairs of midventral cirri subtended by a U-shaped group of 11-12 transverse cirri, 2 large frontal cirri that extend from a transparent cephalized region, and the paroral membrane is small, partly covered by a right buccal lip. This small paroral membrane associated with a buccal lip may be a peristomial cirrus/buccal cleft homologue. The position of frontal cirri, cell shape, and behavior further indicate possible homology. Although these intriguing similarities in H. discocephalus need further study, the above information suggests the discocephalines may have shared close common ancestry with the urostylines.

In the search for what ciliate group may have diverged from a discocephaline lineage, the Euplotina appear as being the most plausible. Like the advanced discocephalines, most euplotines possess a rigid cortex, an ovoid cell shape, hypertrophied frontal cirri, caudal cirri that develop from rightmost dorsal kineties, a reduced left marginal cirral row with right margin cirri absent. Some species of euplotines are obligate psammolittoral forms; although most species have 1 or 2 macronuclei, one species of marine interstitial Diophrys is multimacronucleate: D. multimacronucleatus.

Euplotines exhibit a series of morphogenetic characters (see HILL, 1981) that appear divergent from discocephalines. Both groups, however, begin morphogenesis in a single developmental zone.

Such similarities in morphology and morphogenesis may be products of parallel evolution in 2 unrelated ciliate groups under similar selective pressures (i.e. the marine interstitial) with the same geometric constraints of a shortened, oval cell shape. Alternatively, these shared characteristics may indicate a close phylogenetic relationship between the euplotines and discocephalines, thereby demonstrating a monophyletic origin of the otherwise phylogenetically enigmatic Euplotina.

Nonmenclature and Classification

In 1979 JANKOWSKI proposed the superfamily Discocephaloidea with 2 families, the Amphisiellidae and the Discocephalidae. According to JANKOWSKI, the Amphisiellidae included A. lithophora FAURÉ-FREMIET, 1954 and A. faurei DRAGESCO, 1963. He divided the Discocephalidae into 2 subfamilies: the Discocephalinae, including D. ehrenbergi, D. rotatorius, Erionella macrostoma (syn. Keronopsis macrostoma DRAGESCO, 1963) and Stenotricha arenicolus (syn. Strongylidium arenicolus DRAGESCO, 1953), and the Marginotrichinae, including Marginotricha grandis (syn. Discocephalus grandis DRAGESCO, 1954) and Prodiscocephalus minimus (syn. Discocephalus minimus DRAGESCO, 1968).

Because of significant morphological and morphogenetic differences, P. faurei and P. lithophora clearly do not belong in the same genus as A. marioni. A. marioni is the type species for the genus Amphisiella GOURRET and ROESER, 1887. The names of families are derived from the stem of their type genera; under the Law of Priority, when a family is split, the new subgroups are to be assigned the oldest familial name available, but "the fragment containing the type genus of the original single family must retain the original familial name"

(CORLISS, 1962). Therefore, the Amphisiellidae, as proposed by JANKOWSKI, must be rejected. Because of developmental and morphological homologies, I consider P. faurei and P. lithophora as members of the genus Psammocephalus.

Stenotricha (syn. Strongylidium arenicolus DRAGESCO, 1953), represented by a single species description, bears little similarity with Discocephalus. Until new information deems otherwise, I consider its systematic position uncertain and its placement with Discocephalus-like hypotrichs unjustified.

Erionella (syn. Keronopsis macrostoma DRAGESCO, 1963) appears to have such Discocephalus-like characters as 2 differentiated groups of left marginal cirri, long dorsal bristles, a row of median (midfrontal?) cirri, and multimacronuclearity; it lacks, however, a marked cephalization and has a different arrangement of transverse cirri and associated cytoskeletal components. Although morphogenetic data is needed to verify apparent homologies, I provisionally include Erionella in the Discocephalina; it is sufficiently divergent from the Discocephalids, however, to warrant separation at the familial level.

The species composing the genus Psammocephalus form a continucus morphological series (phenocline) that culminates in the genus Discocephalus. I consider Psammocephalus, along with the more divergent Marginotricha and Prodiscocephalus, as belonging to the Discocephalidae.

Diagnosis of the Discocephalina, its Families and Genera

Order HYPOTRICHIDA STEIN, 1859

Suborder Discocephalina (n. subord.)

Diagnosis. Thigmotactic, multimacronucleate, obligatory psammolittoral forms with marked cephalization (lacking in Erionella); somatic ciliature includes rows of long (7 μm) dorsal bristles and left marginal cirri divided into anterior and posterolateral groups; right marginal cirri may be absent; a median row (reduced in some species) of midfrontal cirri differentiate, during morphogenesis, from a longitudinal series of oblique streaks.

Family Discocephalidae JANKOWSKI, 1979

Diagnosis. Strongly cephalized hypotrichs ranging from elongate to oval in cell shape; in addition to varying numbers of midfrontal cirri, 7 other frontal derivatives may be present: a short series of right frontal cirri, anterior, malar, migratory and accessory transverse cirri plus transverse cirri arranged in a U-shaped group; both a paroral and peristomial cirrus differentiate from the paroral streak; anterior transverse microtubular bundles unit to form posterior cytoskeletal trunk.

Four genera; Type- Discocephalus HEMPRICH and EHRENBERG, 1831

Genus Discocephalus HEMPRICH and EHRENBERG, 1831

Diagnosis. Ovoid cell shape and rigid pellicle; midfrontal cirri and anterior left marginal cirral row reduced; right marginal cirri absent, extensive cirral hypertrophy; highly complex cytoskeleton;

membrane-bound, microtubular protrusions on cephalized region. Peristomial lobe prominent.

Two species; Type - D. rotatorius HEMPRICH and EHRENBERG, 1831

1. D. rotatorius HEMPRICH and EHRENBERG, 1831
2. D. ehrenbergi DRAGESCO, 1960

Genus Marginotricha JANKOWSKI, 1978

Diagnosis. Complete right and left marginal cirral rows; midfrontal series complete but shifted to cell's right; 3 larger frontals plus 1 paroral cirrus extend from the cephalized region; AZM extends only to anterior of cell; right frontal series absent.

One species; Type - M. grandis (DRAGESCO, 1954) JANKOWSKI, 1978

Genus Prodiscocephalus JANKOWSKI, 1979

Diagnosis. Reduced right and left marginal cirral rows; 6 frontals, 1 paroral cirrus extend from cephalized region; AZM extends only to anterior of cell; right frontal series absent; midfrontal series reduced.

One species; Type - P. minimus (DRAGESCO, 1968) JANKOWSKI, 1979

Genus Psammocephalus n.gen.

Diagnosis. Elongate cell shape; pellicle either rigid (e.g. P. borrori) or supple(e.g. P. faurei); complete rows of midfrontal and right and left marginal cirri; peristomial lobe with peristomial cirrus prominent; AZM extends posteriorly along right lateral border of cell.

Four species; Type - P. borrori n.sp.

1. P. borrori (n.sp.)
2. P. dragescoi (n.sp.)

3. P. faurei (DRAGESCO, 1965) n.comb.

4. P. lithophora (FAURÉ-FREMIET, 1954) n.comb.

Family Erionellidae (n.fam.)

Diagnosis. Cephalization slight; buccal cavity expansive; complete midfrontal series subtended by a J-shaped group of transverse cirri; separate anterior transverse cirral microtubular bundles; 2 right marginal rows, 1 left marginal row.

One genus; Type - E. macrostomum (DRAGESCO, 1963) JANKOWSKI, 1978

Genus Erionella JANKOWSKI, 1978

Diagnosis. Same as above.

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ULTRASTRUCTURE AND CORTICAL MORPHOGENESIS IN THE EUPLOTINE HYPOTRICH *CERTESIA QUADRINUCLEATA* FABRE-DOMERGUE, 1885 (CILIOPHORA, PROTOZOA)

CHAPTER III

ULTRASTRUCTURE AND CORTICAL MORPHOGENESIS IN THE EUPLOTINE HYPOTRICH CERTESIA QUADRINUCLEATA FABRE-DOMERGUE, 1885 (CILIOPHORA, PROTOZOA)

Introduction

The suborder Euplotina JANKOWSKI represents a diverse and evolutionarily advanced assemblage that, based on previous studies (HILL, 1981; WICKLOW, 1982), I believe comprises the following genera: Aspidisca, Certesia, Cirrogaster, Cytharoides, Diophrys, Euplotaspis, Euplotes, Euplotidium, Gastrocirrus, Paraeuplotes, and Uronychia. Their evolution reflects radiation into a wide variety of marine, freshwater, and terrestrial habitats. Interstitial, benthic, and planktonic species may occur sometimes within a single genus (for example: Diophrys irmgard, interstitial; D. scutum, epibenthic; D. histrix, planktonic.

Since BORROR's revision of the Hypotrichida in 1972 (BORROR, 1972), many investigations, particularly morphogenetic and ultrastructural studies, have increased our knowledge of hypotrich evolution and phylogeny (BORROR, 1979; BORROR and EVANS, 1979; BORROR and WICKLOW, 1981; CULBERSON, 1981; HILL, 1981; WICKLOW, 1979, 1981a). Within the Euplotina, studies of cortical development (HILL, 1981) and ultrastructure of cyst formation (WALKER and MAUGEL, 1980) in Diophrys as well as ultrastructural and cytochemical studies of Euplotes (BOHM and HAUSMANN, 1981; HAUSMANN and KAISER, 1979; RUFFOLO, 1976a) have been valuable in the determination of homologous structures and morpho-

genetic patterns. Developmental, structural, and functional studies provide a basis for a greater understanding of the adaptive significance and evolution of form.

The present paper is a study of a marine interstitial euplotine: Certesia quadrinucleata FABRE-DOMERQUE, 1885; since its original description (FABRE-DOMERQUE, 1885) this genus has been reported only rarely (SAUERBREY, 1928; VACELET, 1960). I describe the pattern of cortical development during cell division and interphase ultrastructure including organization of various microtubular structures, the cytostome, mucocyst-like inclusions, and a bulbous organelle. I also discuss the possible roles of microtubules in mediating positioning of organellar complexes during morphogenesis. Finally, I use the above results as a means of phylogenetic comparison of Certesia with other hypotrichs.

Materials and Methods

I isolated Certesia quadrinucleata from coarse sand of both Nantucket Island, Massachusetts and Sea Point Beach, Maine, U.S.A. I cultured populations on the diatom Navicula pelliculosa in 25% seawater at 16°C.

Using light optical microscopy, I observed cells live as well as stained using a modified protargol technique (WICKLOW, 1981a). I fixed cells for electron microscopy using Karnowski's Fixative (KARNOWSKI, 1965), 30 min., followed by postfixation in 2% O_sO_4 adjusted to 900 mOs with sucrose, 30 min. My procedure for processing cells for S.E.M. and T.E.M. are described elsewhere (WICKLOW, 1981a). I viewed cells using a JEOL 100s T.E.M. or an AMR 1000 S.E.M.; I examined elemental composition of cell structures using energy dispersive x-ray spectroscopy operated through an AMR 1000 S.E.M.

All references to the cell will be relative to the cell's left or right; in ventral aspect the cell's left corresponds to the reader's right.

Results

Morphology

The general cortical anatomy of Certesia quadrinucleata is shown in figures 1-3. Certesia is an ovoid ciliate, 54-78 µm long (x=67, n=50), 34-53 µm wide (x=44, n=50), with a rigid cortex. The ventral surface is flattened; the dorsal surface is convex. Whereas the posterior end of the cell is rounded, the anterior end is oblique, sloping from left to right with the anteriormost (right) portion forming a cortical cleft. A bulbous organelle, the condylopallium (described below), protrudes from the cortical cleft.

Frontal ciliature comprises a latitudinal series of 5 transverse cirri and 2 oblique ranks of anterior frontal cirri and 1 paroral cirrus. Buccal ciliature includes membranelles and a paroral membrane. Somatic ciliature consists of 6-7 left marginal cirri and 5 dorsal kineties.

Two large microtubular bundles extend from the 2 rightmost transverse cirri to the distalmost collar membranelle; smaller microtubular bundles from the remaining transverse cirri course obliquely to unite with the larger bundles (fig. 1). The left wall of the buccal cavaity is lined with microtubules which descend into the buccal cavity from the membranelles to the right buccal wall (as observed in protargol stained cells).

Four macronuclei are present: two along the right side and two along the left side of the cell. A micronucleus is associated with the anterior, left macronucleus (fig. 1).

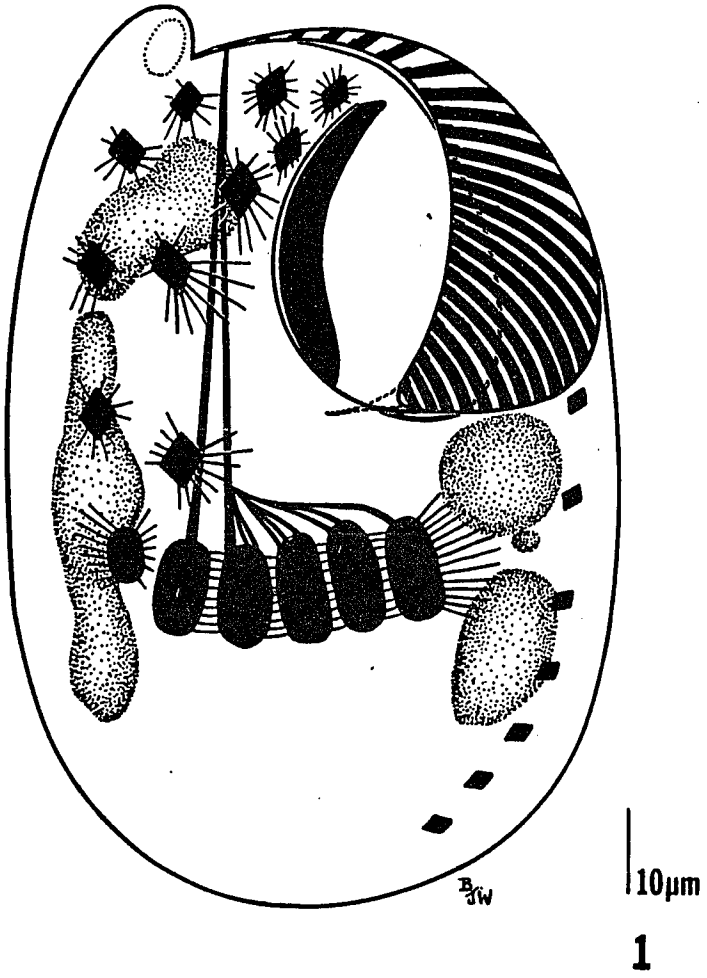
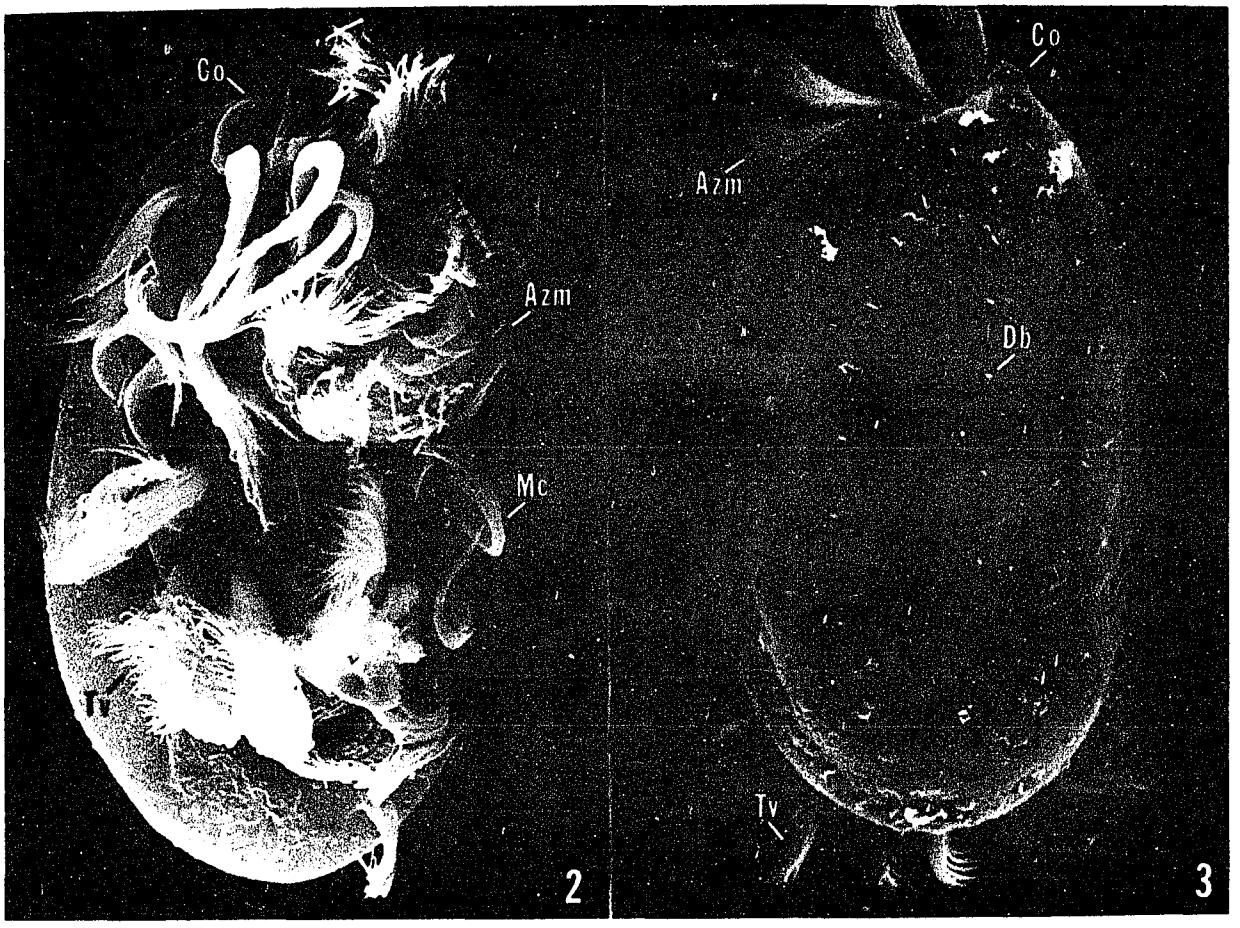


Fig. 1. Ink drawing of Certesia quadrinucleata based on a protargol stained specimen (ventral aspect). Blackened areas represent bases of ciliary structures, black lines represent microtubular bundles, nuclear structures are stippled.

Fig. 2, 3. Scanning electron micrographs of non-dividing cells. 2. Ventral aspect: Azm, adoral zone of membranelles; Co, condylopallium; Mc, marginal cirri; Tv, transverse cirri. X 1600. 3. Dorsal aspect: Db, dorsal bristles. X 1700.


Co
Azm
Mc
Tr
2
Co
Azm
Db
Tv
3

Ultrastructure

Cortex. The cortex of Certesia includes the plasma and alveolar membranes subtended by longitudinal sets of microtubules; electron dense materials (alveolar plates) are found within the alveoli. Alveolar plates, 40 μm thick, are composed of layers of varying electron densities. These plates envelope the whole cell except in regions surrounding organelles or organellar complexes that emerge from indentations of the cortex: cirri, dorsal bristles, the paroral membrane and membranelles, and the condylopallium.

Buccal apparatus. The 25-27 falciform membranelles in Certesia are paramembranelles; each comprises four rows of kinetosomes: the first (posteriormost) and second are of equal length and are the longest rows (approximately 35 kinetosomes), the third row is shorter (by two kinetosomes), and the fourth row is shortest, consisting of three kinetosomes. Kinetosomal rows are longest in the mid-lapel region of the membranellar zone. Transverse microtubules are associated with the kinetosomes of rows three and four (fig. 4), while postciliary microtubules are associated with the first kinetosomal row and the rightmost kinetosomes of the remaining rows (figs. 4, 5, 6). Nematodesmal microtubules descend into the cytoplasm from the base of membranellar kinetosomes (fig. 5).

Two intermembranellar ridges lie between adjacent membranelles; each membranelle is bordered proximally by a short (0.5 μm) ridge and distally by a longer (1.0 μm) ridge (fig. 5). An alveolar plate is continuous through both ridges. The alveolar plate ends at the top of each ridge while the alveolar membranes continue to descend the membranellar face of the ridge, then end just before the mem-

Fig. 4-6. Organization of the membranelles. 4. Transverse section through membranelles each with four (numbered 1-4) kinetosomal rows and separated by intermembranellar ridges (Imr); positions of transverse (T) and postciliary (Pmt) microtubules as well as dense, rod-shaped vesicles (V) are evident. X 33,600. 5. Sagittal section through membranelles showing positions of intermembranellar ridges (Imr), postciliary (Pmt) and nematodesmal (Ne) microtubules. Rod-shaped vesicles (V) attach directly to the cell membrane. X 26,360. 6. Section through proximal membranelles showing postciliary microtubules (Pmt), pharyngeal discs (Pd), and vesicles (V). X 19,260.

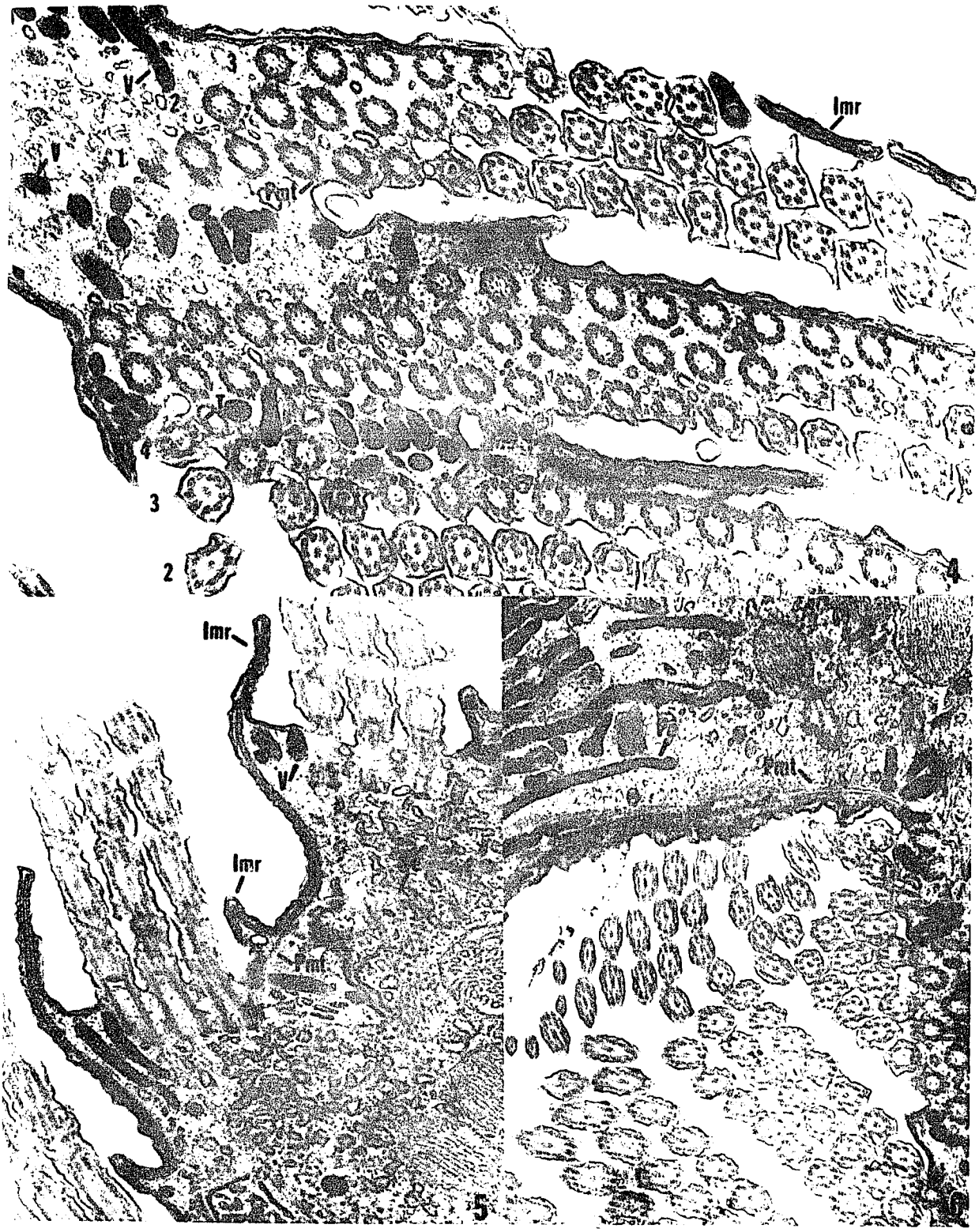

lmr
lmr
lmr
3
2
4
5
6

Fig. 7. Section through the cytosotme. An opening in the septum (S) leads to an atrium (At) with lamellar projections (L); the atrium leads to a constriction bordered by dense striated material. The plasma membrane balloons from this area which is the presumed site of food vacuole formation (Vc). A series of pharyngeal discs (Pd) as well as food vacuoles (Fv) at different stages of digestion are visible. X 16,700.


Ptl
S
At
L
Vc
7

Fig. 8. Section through proximal membranelles showing pharyngeal discs (Pd) separated by postciliary microtubules (Pmt) from the rightmost membranellar kinetosomes. Nematodesmal microtubules (Ne) and vesicles (V) are also present. X 30,400.

Fig. 9, 10. Serial sections through the paroral apparatus. The paroral cirrus (Pc) extends from a cortical shelf beside the oblique series of kinetosomes composing the paroral membrane (Pm). Cross sectioned rod-shaped vesicles (V) line the left side of the paroral membrane; the distal ends of the vesicles are visible beside the paroral cirrus in fig. 9 and are visible in full cross section in fig. 10. The buccal cavity (Bc) is at the lower right in each micrograph. X 21,000.

9 10

branellar cilia. Postciliary microtubules from row one membranellar kinetosomes extend past the proximal intermembranellar ridge to the distal intermembranellar ridge of the adjacent membranelle (fig. 5).

Osmophilic pharyngeal discs (food vacuole membrane precursors) line the right side of the cytopharynx near the cytosotme (figs. 6, 7, 8). Groups of pharyngeal discs are arranged in linear arrays, perpendicular to the cytopharynx by microtubules. These microtubular bundles apparently originate as postciliary microtubules from only the rightmost kinetosomes of each membranelle (figs. 6, 7, 8). In addition to pharyngeal discs, rod-shaped electron dense vesicles border the buccal cavity (figs. 4-8).

At the proximalmost region of the buccal cavity a septum extends into the cytopharynx; within this septum is an oral opening leading to an atrium (fig. 7). Oral lamellae project into the atrium which leads to a 0.4 μm constriction bordered by striated material. The cytostomal membrane balloons out from this constriction which I presume to be the site of food vacuole formation.

The paroral membrane (a polystichomonade) consists of a longitudinal series of approximately 60 oblique kinetosomal arrays with approximately 10 kinetosomes in each array (figs. 9, 10). A paroral cirrus extends from a cortical shelf just right of the anterior paroral membrane. Groups of electron dense visicles border the right side of both the paroral cirrus and the paroral membrane (figs. 9, 10). An endoral membrane is absent.

Cirri. Most frontal cirri arise from parallelogram-shaped packets of kinetosomes (fig. 11). Kinetosomes of transverse cirri, however,

Fig. 11. Cross section through a frontal cirrus (anterior is at the left, posterior is at the right of the micrograph). A gradation is evident in vesicles (V) in both degree of electron density and in shape. Large microtubular bundles (Mb) extending anteriorly from transverse cirri as well as nematodesmal (Ne) and subcortical (Cmt) microtubules are evident. X 38,900.

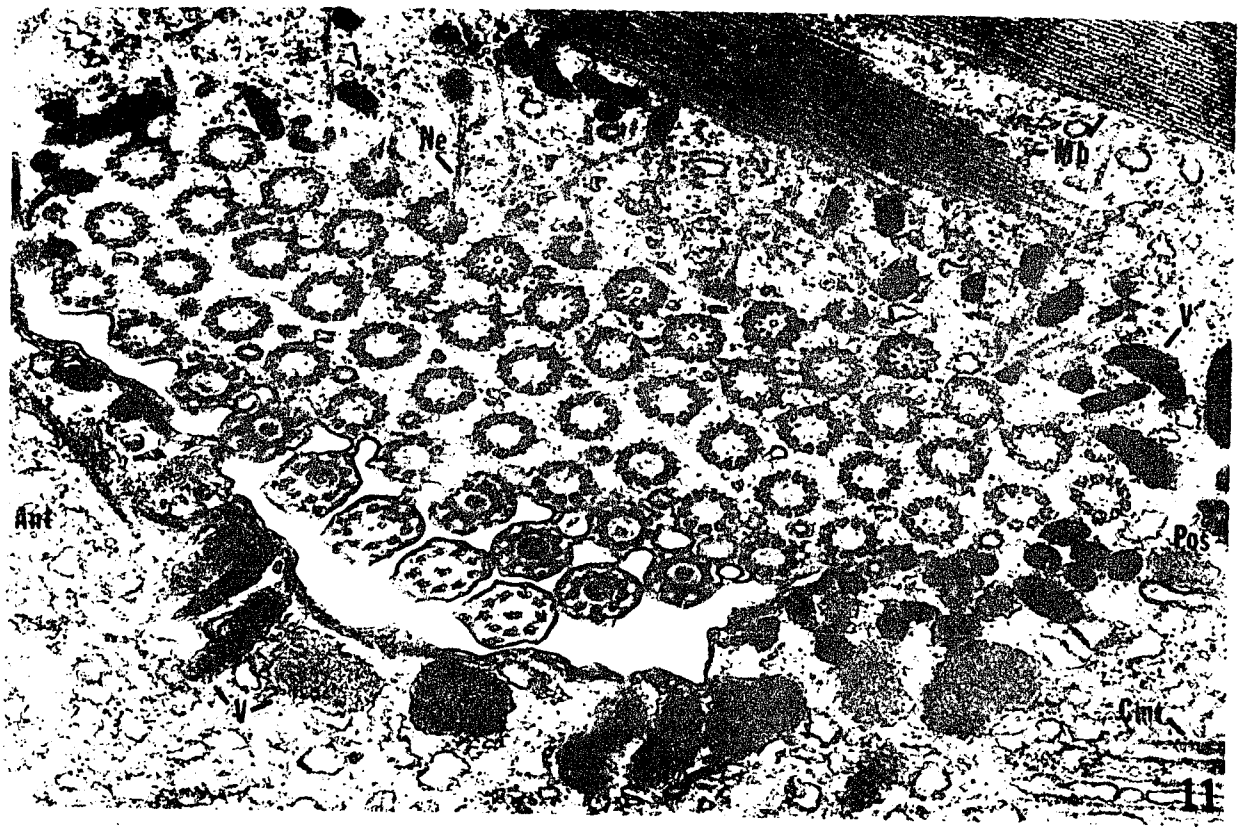
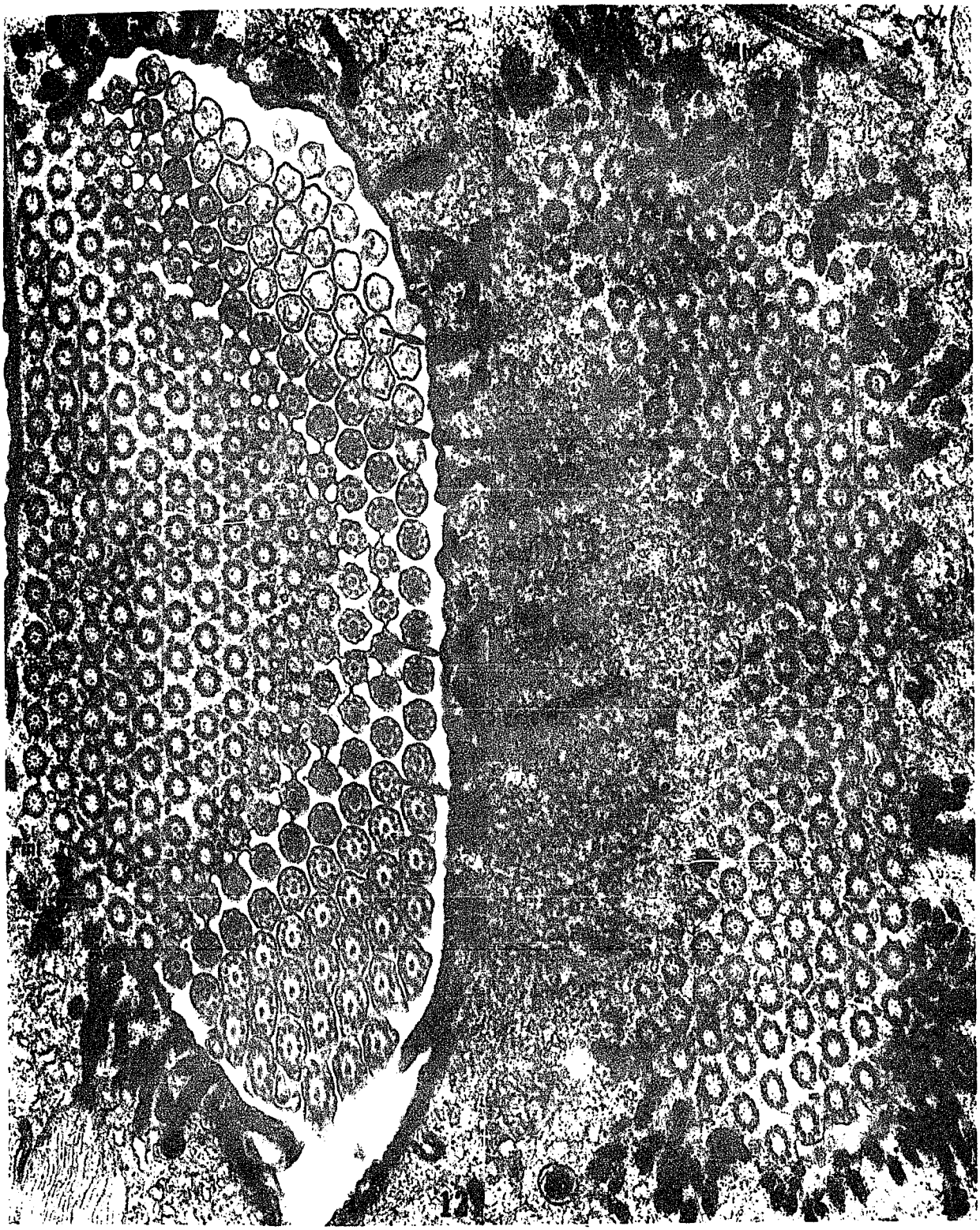

Ne
Ant
V
Pos
V
Cut
11

Fig. 12, 13. Serial sections through transverse cirri showing the arrangement of rod-shaped vesicles (V) beside the cirrus: vesicles attach directly to the plasma membrane (arrows). A microtubular bundle from a left transverse cirrus and nematodesmal (Ne) and postciliary (Pmt) microtubules are visible. X 26,000.

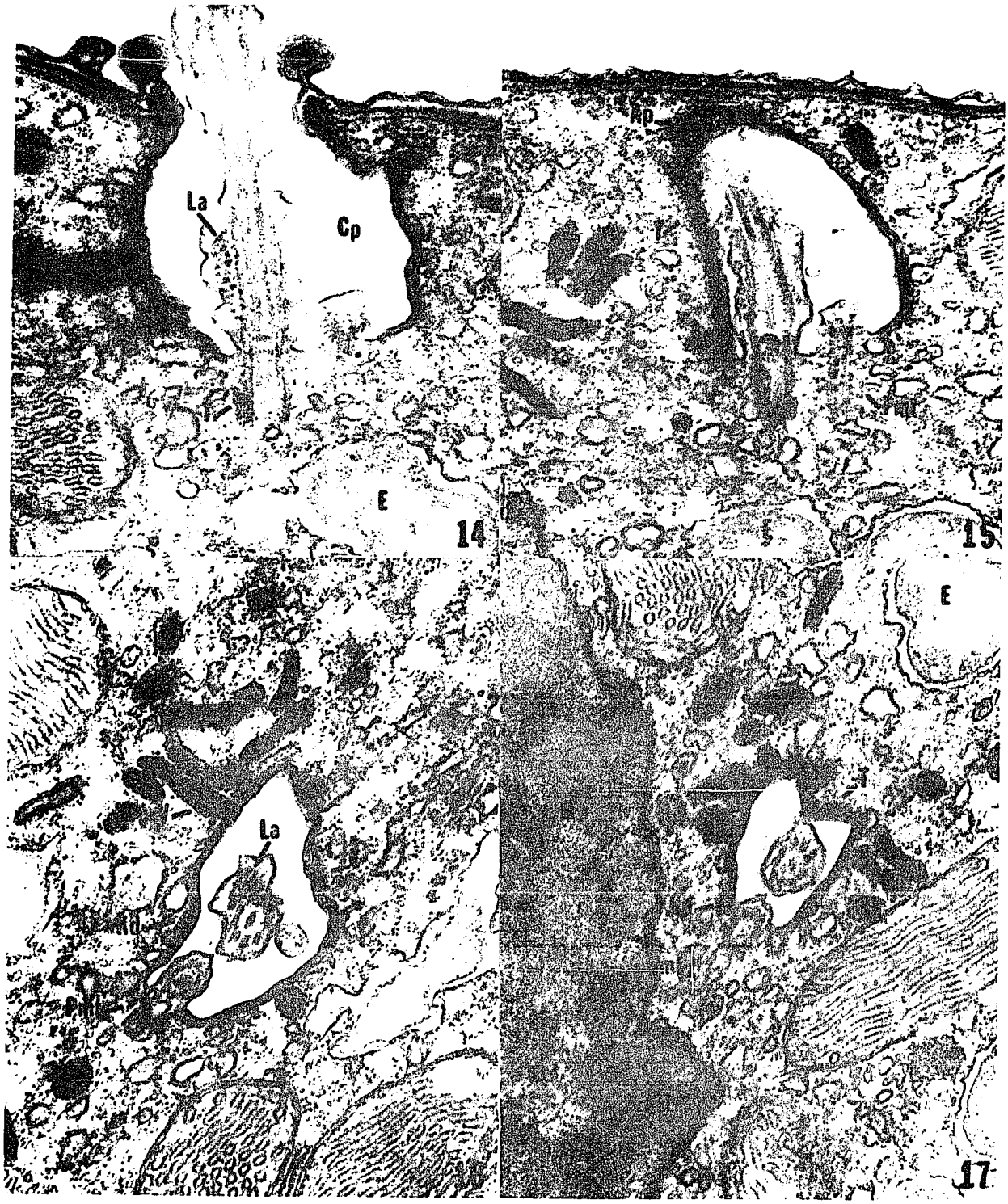


are arranged in the form of an irregular polygon; each hypertrophied cirrus comprises approximately 280 kinetosomes (figs. 12, 13). Postciliary microtubular ribbons originate at the righ posterior kinetosomes of each transverse cirrus while single postciliary microtubules arise from internal kinetosomes (figs. 12, 13). Two large microtubular bundles arise from each of the 2 rightmost transverse cirri and extend toward the collar membranelles (figs. 1, 11). Smaller microtubular bundles (figs. 1, 13) from the 3 leftmost transverse cirri course obliquely to join with the large rightmost bundles. In this way microtubular bundles of all 5 transverse cirri are united as a major endoskeletal component. Nematodesmal microtubules radiate from the base of cirral kinetosomes (fig. 11).

Two kinds of electron dense vesicles are associated with frontal cirri (figs. 11-13). Most common are rod-shaped vesicles (0.8 μm long, 0.15 μm wide) arranged with their long axis perpendicular to the cell membrane; they appear to make direct contact with this membrane. The second kind of vesicles appear less dense and are irregularly shaped spheres (0.4 μm wide). These spherical vesicles may represent stages in the formation or the dense rod-shaped inclusions (fig. 11).

Dorsal bristle complex. Each dorsal bristle complex consists of a pair of kinetosomes. The anterior member of the pair is positioned approximately 60° to the right of the posterior member, relative to the long axis of the cell; the anterior kinetosome is ciliated, the posterior kinetosome is nonciliated (a condylocilium). Both kinetosomes lie within a 1.3 μm deep cortical pit (fig. 14). The bristle cilium protrudes 1.5 μm above the pit opening - all bristle

Fig. 14-17. Structure of the dorsal bristle complex. Legend: Ap, alveolar plate, Cp, cortical pit; E, endosymbiont; Kd, kinetodesmal fiber; La, lasiosomes; Ma, macronucleus; Pmt, postciliary microtubules; T, transverse microtubules; V, rod-shaped vesicles. X 36,000.


La
Cp
E
14
Ap
15
E
La
17

cillia are the same length.

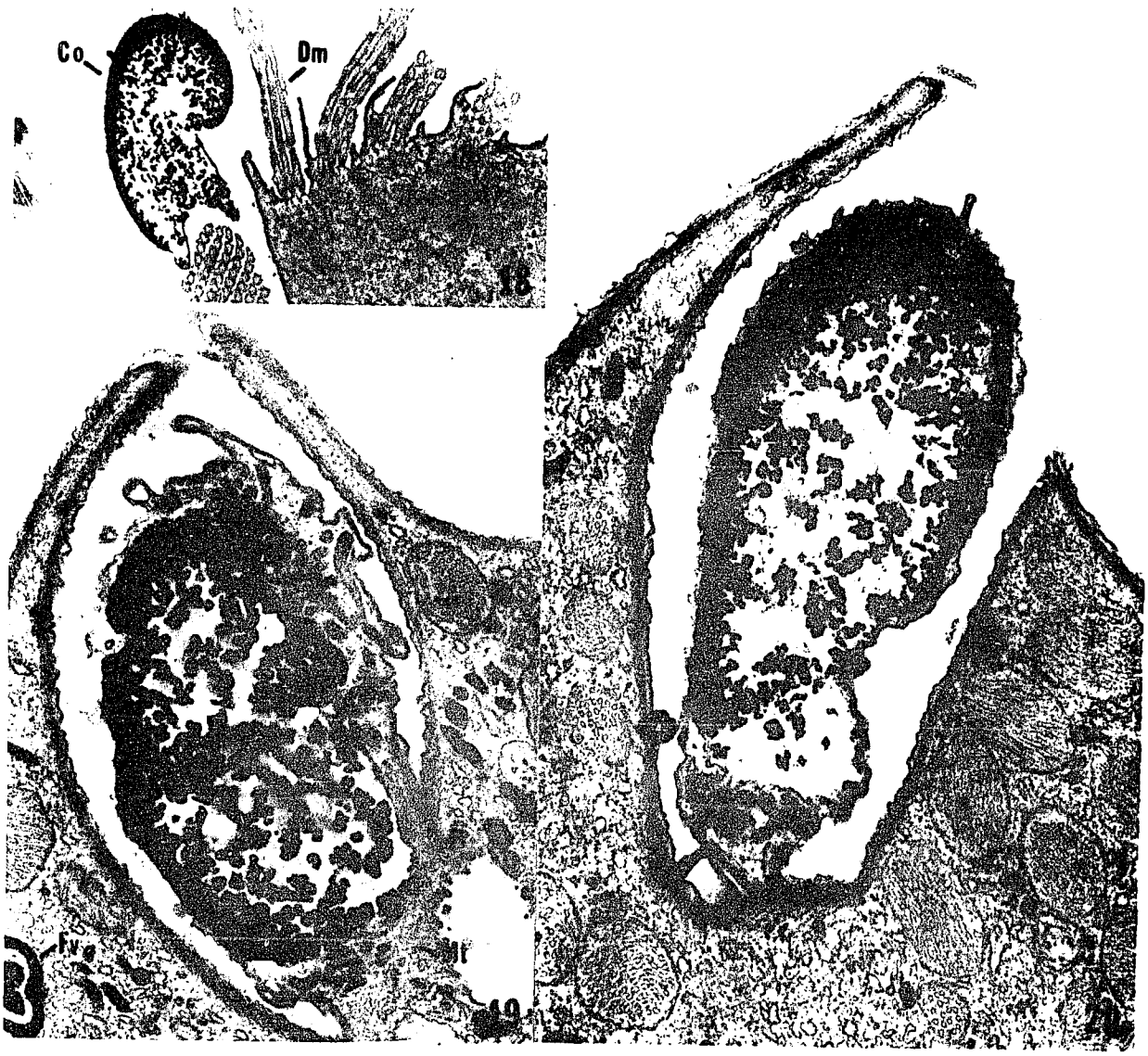
The pit opening is surrounded by a ring of fibrous plate material within the alveolus; although the alveolar plate ends at the pit opening, the alveolar membranes descend the pit wall, then end near the base of the cilium and condylocilium (figs. 14, 15). A transverse microtubular ribbon comprising 8 microtubules originates on the anterior kinetosome, while a postciliary microtubular ribbon, consisting of 3-4 microtubules, originates on the posterior kinetosome (figs. 14-17). Both microtubular ribbons extend toward the pellicle. A kinetodesmal fiber originates near triplets 7 and 8 of the posterior kinetosome, then runs anterior at an oblique angle toward the pellicle. Other kinds of fibrillar material associated with the bristle complex, such as found in Discocephalus (WICKLOW, 1982), are absent in Certesia.

A cluster of 46 nm, electron dense particles - lasiosomes - are contained within a space between the axoneme and ciliary membrane on the anterior face of the bristle cilium (figs. 14, 16). Bristle lasiosomes are located just within the cortical pit; they appear to be grouped in at least 3 linear arrays.

Dense rod-shaped vesicles are also associated with the bristle pit plasma membrane; endosymbiotic bacteria are present (figs. 14-17).

Condylopallium. An ovoid structure (7.5 μm long, 3.7 μm wide) bulges ventrally and extends anteriorly from a cleft located at the right, anteriormost end of the cell, juxtaposed to the distalmost collar membranelle (figs. 2, 3, 18). The bulb is enveloped by both plasma and alveolar membranes; alveolar plates are absent. A narrow stalk (which widens dorsally) supports the bulb within the cortical cleft (figs. 19, 20).

Fig. 18-20. Structure of the condylopallium (see also figs. 2, 3). Legend: Co, condylopallium; Dm, distal membranelle; Fv, food vacuole; Mt, microtubules. Intense electron dense material within the condylopallium is composed primarily of silicon. 18. Condylopallium juxtaposed to the distal collar membranelles. X 16,100. 19. Section through the dorsal region of the condylopallium showing cytoplasm intercalating with the siliceous core. X 28,700. 20. Condylopallium extending from a stalk within the cortical hood. X 19,400.


Co
Dm
Ivc
lt

Within the core of the bulb is a membrane-bound organelle composed of an electron dense periphery and electron dense particles dispersed through an electron lucid interior. Cytoplasm, containing dense, rod-shaped vesicles, surrounds (and sometimes intercalcates with) this organelle posteriorly and dorsally (figs. 19, 20). I have observed several very dense vesicles with electron lucid lumens within the cytoplasm near this bulb (fig. 19). These kinds of vesicles also appear in the cytoplasm near the cytostome; I presume them to be food vacuoles containing diatoms at different stages of digestion. Microtubules extend anteriorly into the cytoplasm that surrounds the bulb (fig. 19). Their origin and terminus are unknown.

A comparative elemental analysis of the condylopallium and other parts of the cell, showed no significant differences in elemental composition. Although the cells were fed exclusively on diatoms, silicon was not detected from either the condylopallium, the remaining portion of the cell, nor from clusters of diatoms themselves.

The term condylopallium refers to the protrusion of the organelle from the cell, and its partial covering by a cortical hood (condylo = knob, prominence; pallium = mantle, coverlet).

Morphogenesis

Cortical morphogenesis in Certesia begins in a single latitudinal developmental zone. The first cortical event within this zone is the de novo development of the opisthe oral primordium (OP) within a subsurface pouch. Initial kinetosomes appear as a dense cluster on the dorsal surface of the pouch. As kinetosome proliferation

continues, pouch enlargement ensues accompanied by promembranelle formation; membranelles differentiate from right to left and posteriorly. During this process, which continues until equatorial cleavage furrow is formed, the developing membranellar zone becomes slightly twisted into a descending spiral: a reservoir of kinetosomes lies at the base of this spiral while distally (anteriorly) maturing membranelles erupt onto the cell surface (fig. 21 a-l).

Meanwhile, the left side of the parental lapel partially dedifferentiates such that membranellar cilia generally shorten, while most membranellar kinetosomes remain undifferentiated and in their original alignment. The parental membranelles are then inherited by the proter.

Five frontal primordia (FP) appear during OP development. FP initiation proceeds in a left to right developmental gradient across an initially single, latitudinal zone. The 3 leftmost FP appear first; each arises as a trickle of kinetosomes beside a longitudinal cortical ridge (fig. 21a) The cortical ridges are anterior extensions of ridges bordering transverse cirri. Soon after the FP arise, longitudinal fibrils (presumably microtubular bundles) appear along both sides of each developing FP (fig. 21b). Eventually all 5 frontal streaks are bordered on both left and right sides by these microtubular tracks; the tracks are attached posteriorly to cirri: the first (leftmost) 3 tracks attach to the first (leftmost) transverse cirri, the fourth track is attached to the fifth transverse cirrus (the fourth transverse cirrus is skipped), and the fifth track is attached to the right, posteriormost frontal cirrus (fig. 21 b-f).

After the first 3 FP have begun to elongate as frontal streaks, the fourth FP appears beside the leftmost, posterior, frontal cirrus

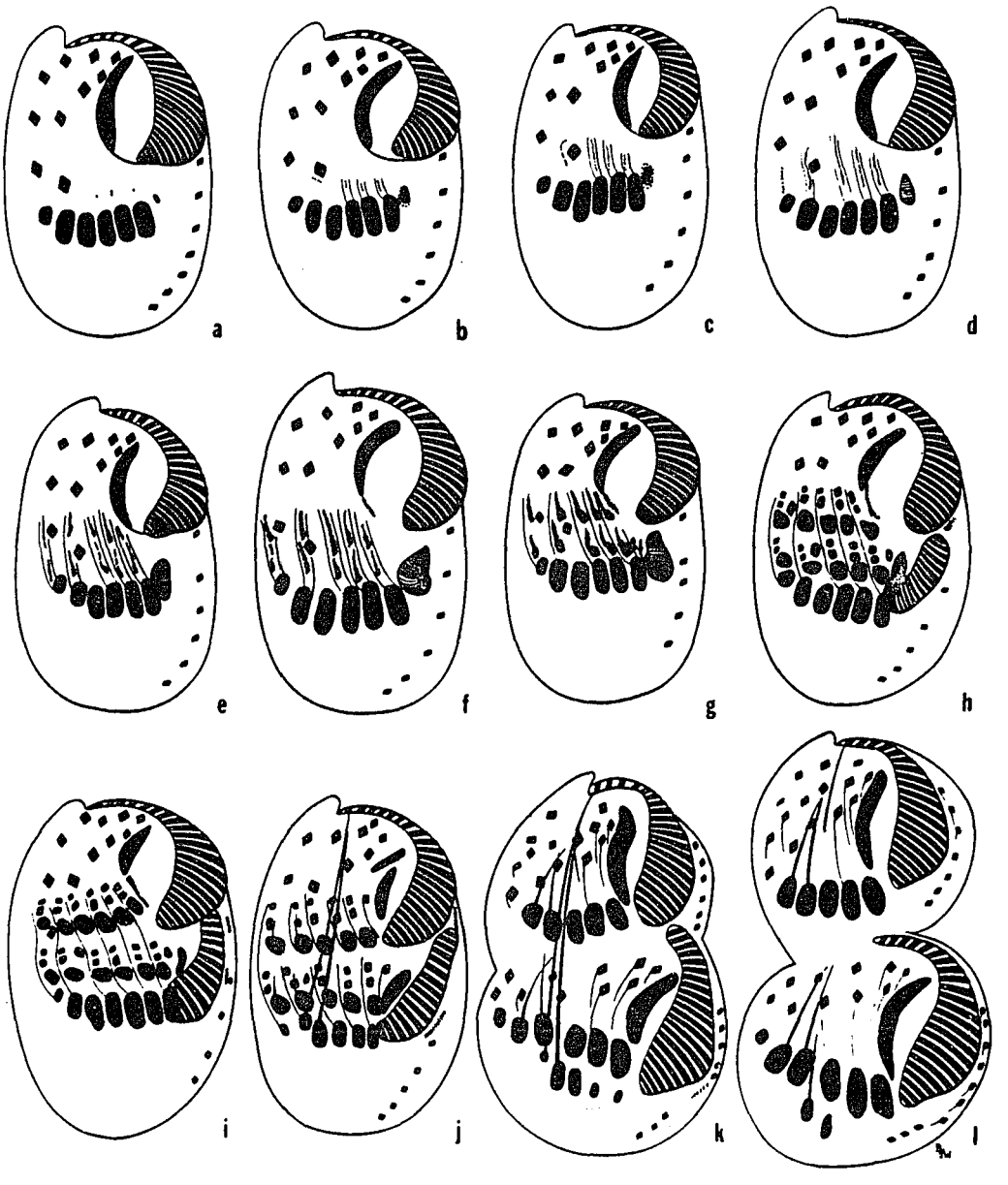


Fig. 21 (a-l). Line diagrams based on protargol stained specimens depicting a sequence of ventral, cortical morphogenetic stages during cell division. Black areas represent ciliary organelles, black lines represent microtubular structures.

(fig. 21b). Still later, the fifth and final FP develops next to the rightmost, posterior frontal cirrus. Although all FP appear to arise de novo, each has a close positional relationship with a parental cirrus. A concomitant lengthening of the microtubular tracks beside each frontal streak accompanies streak elongation.

As the 5 frontal streaks lengthen, they divide into 2 sets, thereby forming proter and opisthe frontal fields. Lateral kinetosome proliferation, forming a linear series of oblique, kinetosomal arrays from the initial kinetosomal pairs within each streak, accompanies this division. At this time, the left microtubular track beside each frontal streak disappears while each right track continues to lengthen (fig. 21g).

Each frontal streak matures in a posterior to anterior direction: cilium growth begins on the posteriormost streak kinetosomes while the anteriormost streak kinetosomes are still kinetosomal pairs. Thus transverse cirral anlagen mature (both in lateral kinetosome proliferation and ciliation) first, followed by the anterior 2 additional cirral anlagen within each streak. The cirral anlagen then separate forming 3 latitudinal ranks of procirri within both proter and opisthe fields; as proter and opisthe fields separate, microtubular tracks split into anterior (proter) and posterior (opisthe) components (fig. 21j).

While procirri enlarge and begin to migrate toward their interphase cortical positions, a large microtubular bundle appears on the anterior edge of each of the 2 rightmost transverse procirri in both proter and opisthe frontal fields (fig. 21j). The assembly of these microtubular bundles proceeds anteriorly. At the same time,

the 2 anterior ranks of procirri in each field also migrate anteriorly until they are positioned obliquely; the posteriormost rank (transverse procirri) remains latitudinal.

During the final stages of procirral migration the microtubular tracks assume a positional modification: they now appear linked to the posterior margin of the 2 anterior ranks of procirri while connected posteriorly with the transverse procirri (fig. 21j,k). In both the proter and opisthe, each microtubular track appears as 2 segments with the anterior end of each segment connected to a procirrus. The posterior end of the microtubular track is now attached to the anterior border of transverse procirri: the original nexus between the microtubular track and the parental transverse cirri is lost. The order of this sequence of events is unknown. The microtubular bundles disappear after procirri are in their final interphase position.

When the frontal streaks begin to separate into 2 frontal fields, the posterior region of the paroral apparatus dedifferentiates; this process continues anteriorly, followed by the formation of a proter paroral primordium with apparent utilization of kinetosomes from the dedifferentiated parental paroral membrane. At this time an opisthe paroral primordium develops from the right side of the opisthe OP. A paroral cirrus differentiates from each developing paroral primordium.

During differentiation of the paroral primordia, 2 additional kinds of primordia appear: marginal cirral primordia (Mcp) and dorsal bristle primordia (Dbp). The anterior (proter) Mcp if formed by the dedifferentiation of the anteriormost left marginal cirrus; sub-

sequently, a posterior (opisthe) Mcp forms beside the next posterior, marginal cirrus (fig. 21h, i). From each of these anlagen differentiates 6 or 7 marginal cirri. Microtubular bundles appear on the posterior border of each marginal procirrus during their migration to interphase positions (fig. 21 l). These microtubular bundles are present only during morphogenesis.

At the time of procirral formation, Dbp appear as pairs of kinetosomes associated with parental dorsal bristles. All but the anteriormost and posteriormost dorsal bristles contribute Dbp. Parental bristle complexes retain their cilia during new kinetosome proliferation; dorsal bristle development proceeds within cortical grooves.

Parental cirri are gradually dismantled and resorbed during the late stages of division. Parts of parental cirral structures (including the large microtubular bundles attached to right transverse cirri) persist through cytokinesis (fig. 21 l).

Discussion

Ultrastructure

Cortex. The alveolar plates of Certesia are similar to those described in Euplotes (HAUSMANN, 1978; RUFFOLO, 1976a). In both Euplotes and Certesia the plates have a distinct substructure: layers of electron dense material interspersed between electron lucid layers. Cytochemical studies indicate alveolar plates in Euplotes to be composed of protein with a fine coating of polysaccharide (BOHM and HAUSMANN, 1981). Due to organization similarity, I consider alveolar plates in Certesia and Euplotes to be homologous. The plates appear to contribute to cell rigidity.

Rod-shaped electron dense vesicles are arranged with their long axis perpendicular to the plasma membrane in regions where the alveolar plates are absent: at the base of cirri, membranelles and dorsal bristles. The vesicles are membrane-bound and are composed of a homogeneous material; they appear to contact the plasma membrane in areas devoid of alveoli. Dense vesicles have also been observed near the cirral bases of Euplotes (GLIDDON, 1966: ROTH, 1957); these appear larger and less dense than those in Certesia. During encystment in Diophrys, dense vesicles (cyst wall precursors) fuse to the plasma membrane at the base of cirri, at the oral region, and at the base of dorsal bristles (RUFFOLO, 1976a). Fusion of vesicles to the plasma membrane in Certesia, Euplotes, and Diophrys occurs in regions where alveolar plates are absent. Although further study is needed, I believe the dense vesicles in Certesia may be muciferous bodies,

as defined by HAUSMANN (1978), and function as cyst wall precursors.

Buccal apparatus. JERKA-DZIADOSZ (1980) in an ultrastructural study of stomatogenesis in Paraurostyla weissei, demonstrated the formation of the endoral membrane from the left side of the developing paroral primordium; a paroral cirrus differentiates from the anterior end of the primordium. Hypotrich paroral membranes are composed of one to many rows of kinetosomes; endoral membranes, in all hypotrichs studied thus far, consist of one kinetosomal row.

The buccal apparatus of Certesia includes 3 major ciliary components: membranelles, a paroral membrane, and a paroral cirrus; an endoral membrane is absent. Endoral membranes are also absent in the euplotines Euplotes, Euplotidium, and Gastrocirrus. Other euplotines such as Diophrys and Cytharoides as well as hypotrichs in general have both paroral and endoral membranes. Diophrys, because of morphological and developmental characters shared with other hypotrichs, is considered as the most primitive euplotine (HILL, 1981).

Lack of a separate endoral membrane suggests, perhaps, that Certesia retains (by neoteny) a single membrane paroral apparatus; this character may then unite Certesia, Euplotes, Euplotidium, and Gastrocirrus as close relatives. I consider Cytharoides, with a partial endoral membrane, as intermediate between Diophrys and Certesia.

Pharyngeal discs. Disc-shaped, electron dense, food vacuole membrane precursors (KLOETZEL, 1974) have been observed in all euplotines studied with electron microscopy: Certesia (present paper), Diophrys (RAFFAELLI, 1970; WALKER and MAUGEL, 1980), and Euplotes (KLOETZEL, 1974; ROTH, 1957; TUFFRAU et. al., 1968). These packets of membrane are different, both in form and electron density, from

those lining the cytopharynx in non-euplotine hypotrichs such as Discocephalus (WICKLOW, 1982), Stylonychia (PUYTORAC et. al., 1976), and Thigmokeronopsis (WICKLOW, 1981a) and may represent a distinct feature of the suborder.

Pharyngeal discs in Certesia are arranged in an orderly series along the cytostome by postciliary microtubular ribbons which descend along the buccal cavity from the rightmost kinetosomes of lapel membranelles. Pharyngeal discs are thus compartmentalized in parallel arrays. Postciliary microtubules are also associated with the rightmost kinetosomes in lapel membranelles of Paraurostyla weissei (JERKA-DZIADOSZ, 1980); these are thought to be the "terminal fibers" (BAKOWSKA and JERKA-DZIADOSZ, 1978) that also descend the cytopharynx toward the cytostome.

Cirri. The presence of hypertrophied cirri, especially transverse cirri, is generally characteristic of all euplotines as well as some members of the Discocephalina (WICKLOW, 1982). The 2 large microtubular bundles that extend anteriorly from the 2 rightmost transverse cirri to the collar membranelles (along with smaller bundles from leftmost transverse cirri) appear homologous to the microtubular bundles - "rootlet fibers" - of Euplotes (GLIDDON, 1966; ROTH, 1957; TUFFRAU et. al., 1968). Microtubules found between internal cirral kinetosomes of Certesia appear similar to those described in Discocephalus (WICKLOW, 1982).

Dorsal bristle complex. LYNN (1981) has reviewed the organization of microtubular organelles in ciliates. The fundamental structure of the dorsal bristle complex in Certesia concurs with the hypotrich dikinetid pattern characterized by LYNN.

The transverse and postciliary microtubular ribbons and the kinetodesmal fiber of Certesia are similar in position and direction to those described in Euplotes (RUFFOLO, 1976a), Discocephalus (WICKLOW, 1982), and the immature dorsal kinetids of Oxytricha (GRIMES and ADLER, 1976). Both these latter taxa have additional, single post-ciliary microtubules associated with the anterior kinetosome.

As in Euplotes (HAUSMANN and KAISER, 1979; RUFFOLO, 1976a) alveolar plate material surrounds the opening of the bristle pit in Certesia but does not descend to the base of the pit. Although I have not observed a rosette of ampules surrounding the bristle pits in Certesia, there are many muciferous vesicles associated with the pit wall. These make contact with the plasma membrane where alveolar membrane is absent.

The presence of lasiosomes within the bristle cilium of Certesia is the first instance of these possible sensory structures (RUFFOLO, 1976a) observed outside the genus Euplotes. Their position and linear arrangements suggest close homology to those found in Euplotes.

Condylopallium. Supposed statocyst-like inclusions (concrement vacuoles and Mullers vesicles) have been observed in gymnostome and trichostome endosymbionts and various karyorelictid ciliates (CORLISS, 1979) as well as in hypotrichs such as Psammocephalus lithophora (syn. Amphisiella lithophora) and Amphisiella marioni; most of these vesicles appear to contain $CaCO_3$ (FAURÉ-FREMIET, 1954).

Spherical bodies found in the cyst walls of heliozoa such as Echinosphaerium are composed of silicon (PATTERSON and THOMPSON, 1981); the core material within the condylopallium of Certesia appears similar ultrastructurally to these siliceous bodies. I hypothesize

the source of dense material within the condylopallium of Certesia to be undigestable material from tests of ingested diatoms (possibly silicon).

The function of the condylopallium may be simply to serve as a reservoir for the accumulation of undigestable contents of food vacuoles. Under this hypothesis one could predict that after a threshold volume of undigested material was accumulated, the bulb could be pinched off from the cell.

Alternatively, the position of the bulb on a narrow stalk, its enclosure within a cortical cleft, and its location on the anteriormost part of the cell suggest its possible function as a sensory device. One likely sensory role is that of an organelle of equilibrium, a statocyst. The condylopallium within the cortical cleft may prove to be analagous to the statocyst within the hood in the rhopalium of scyphozoan cnidarians. This hypothesis may be tested by removal of of the bulb by microsurgery.

Morphogenesis

Developmental patterns resulting from deployment of incipient organelles or organellar complexes during morphogenesis are higher level developmental events that can be used to identify homologous structures and demonstrate recent common ancestry (BORROR and WICKLOW, 1981: WICKLOW, 1981a). The developmental pattern of cell division morphogenesis in Certesia is homologous to that described in other euplotine hypotrichs: Aspidisca (DEROUX and TUFFRAU, 1965; HILL, 1979), Diophrys (HILL, 1981), Euplotes (RUFFOLO, 1076b), Euplotidium (HILL, 1980), and Uronychia (HILL, 1978). Ciliary primordia arise within a single latitudinal developmental zone as in Discocephalus (WICKLOW, 1982). In Discocephalus however, frontal primordia develop

in close association with the oral primordia (which develops on the cell surface); the oral primordium in Certesia develops separately from frontal primordia and occurs within a subsurface pouch.

During the first stage of division morphogenesis in Certesia, microtubular bundles appear on both sides of each developing frontal streak. This location suggests these bundles may serve as tracks that guide the longitudinal alignment of kinetosomes during streak elongation. Their attachment to parental transverse cirri would provide a stationary channel in which orderly kinetosomal proliferation may occur. At the time of procirral separation and lateral growth, the left microtubular bundle associated with each streak disappears; thus, the right microtubular bundle provides for streak alignment during late division.

Subcortical microtubules are present in both Certesia and Euplotes (GRIM et. al., 1980, 1982; RUFFOLO, 1976a). During morphogenesis in Oxytricha (GRIMES, 1972), these subsurface sheets of microtubules disappear in areas of streak formation, thereby providing a possible cortical channel for growth and migration of cirral anlagen. The same process probably also occurs in Certesia and Euplotes but what changes may occur within the alveolar plates in regions of streak formation needs further study.

The role of the remaining microtubular tracks in Certesia appears to change during procirral migration. Each microtubular bundle loses its connection to the parental transverse cirri; the bundles then attach posteriorly to transverse procirri while connected anteriorly to anterior frontal procirri. Bundles appear to lengthen as procirral migration proceeds. The change of attachment sites, concommitant

lengthening during procirral migration, and subsequent disappearance after final procirral positioning leads me to believe that the microtubular bundles may undergo a change from a passive to a possible active role during morphogenetic movement of procirri.

In an ultrastructural morphogenetic study, JERKA-DZIADOSZ (1980) observed an additional system of microtubules in Paraurostyla weissei: intrastreak microtubules (occurring as oblique arrays between procirri) and basal microtubules (located at the base of each cirral streak). Although arranged differently along the cirral streak, the microtubular tracks of Certesia appear most like the basal microtubules of P. weissei. JERKA-DZIADOSZ hypothesized that microtubules may play a direct role in procirral movement by elongation of microtubules or microtubule to microtubule sliding, or an indirect role by serving passively as microtubular tracks. This study suggests both direct and indirect roles may operate at different times in Certesia. An ultrastructural study of cortical morphogenesis in Certesia is necessary to elucidate the nature of transient microtubules and their possible roles in mediating organelle positioning during development.

Certesia's divergence from Euplotes is shown by its many unique characters: the condylopallium, mucocyst-like vesicles, presence of a complete left marginal row (as in Cytharoides) while caudal cirri are absent, quadripartite nuclear configuration, and the mode of initiation and deployment of frontal primordia. These differences notwithstanding, Certesia bears a remarkable similarity to Euplotes. Both genera share distinct pharyngeal discs, alveolar plates, "rootlet fibers", a single membrane paroral apparatus, similar dorsal bristle organization including lasiosomes, an ovoid (although Certesia is

more squared) cell geometry, and a subsurface pouch stomatogenesis.

In addition to suggesting a close phylogenetic relationship between these two genera, the above similarities connote also a character array that may be useful in justifying the inclusion of other genera within the Euplotina. For example, Swedmarkia has been provisionally appended to the Euplotidae (BORROR, 1972; CORLISS, 1979); comparative ultrastructural and morphogenetic data, such as described above, are necessary to verify the systematic position of this genus.

Nomenclatural Note

In 1960 VACELET (1960) described a second species of Certesia: C. ovata. According to Vacelet this species was distinct from C. quadrinucleata because of variation in shape and mobility of transverse cirri, and in different cell length. Mobility of cirri probably depends on individual variation and the physiological state of the cell. Cell length of C. ovata and C. quadrinucleata overlap; cell length in general may not be used safely to identify species (BORROR and WICKLOW, 1982). Hence, I consider C. ovata as a junior synonym for C. quadrinucleata.

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EPICLINTES AMBIGUUS (MÜLLER, 1786) BÜTSCHLI, 1889 (EPICLINTINA, N.SUBORD.): ULTRASTRUCTURE AND CORTICAL MORPHOGENESIS OF A BIZARRE, MARINE, EPIBENTHIC HYPOTRICH (CILIOPHORA, PROTOZOA)

CHAPTER IV

EPICLINTES AMBIGUUS (MÜLLER, 1786) BÜTSCHLI, 1889 (EPICLINTINA, N.SUBORD.): ULTRASTRUCTURE AND CORTICAL MORPHOGENESIS OF A BIZARRE, MARINE, EPIBENTHIC HYPOTRICH (CILIOPHORA, PROTOZOA)

Introduction

Since its discovery by MÜLLER in 1786, *Epiclintes* has been assigned (at least provisionally or as *incertae sedis*) to several hypotrich families (see below); justification for these systematic decisions was based primarily on the interphase arrangement of ventral cirral rows. As hypotrichs sharing a similarly arranged ventral ciliature may have strikingly different ontogenies, classifications based on mere interphase organization of the ciliature may be artificial BORROR and WICKLOW, 1981). Hence, the phylogenetic position of *Epiclintes* has remained ambiguous.

It is becoming evermore convincing that morphogenetic and ultrastructural information can be used safely as a means to determine homology and demonstrate common ancestry, thereby providing evidence for natural relationships within the Hypotrichida (BORROR, 1979; CULBERSON, 1981; HILL, 1981; JERKA-DZIADOSZ, 1981b; WALKER and MAUGEL, 1980; WICKLOW, 1981b). In my previous studies I have hypothesized a close phylogenetic relationship between the Euplotina, Discocephalina, and Urostylina (WICKLOW, 1982a). This phylogeny is based on the pattern of cortical morphogenesis exhibited during cell division, coupled with ultrastructural data on features of the buccal and somatic cortex. Where does *Epiclintes* fit into this scheme?

In general, an increase in ciliary organelles (polymerization) is thought to be associated with early ciliate evolution; a decrease in ciliary organelles through reduction, fusion, or change in function (oligomerization) is though to be associated with more recent ciliate evolution (see POLJANSKI and RAIKOV, 1976). I therefore hypothesize that the multiple, oblique rows of ventral cirri in Epiclintes would develop from a longitudinal series of oblique frontal streaks. In this way the ventral ciliature of Epiclintes would be so ontogenetically similar to the ventral ciliature in Urostylines such as Thigmokeronopsis (WICKLOW, 1981a) as to be homologous. Under this hypothesis, Epiclintes would represent an early hypotrich divergence from which lineage the urostyline hypotrichs, by adaptive exploitation of a midventral ciliature (via oligomerization of multiple frontal rows), may have evolved.

In this present paper I use cortical morphogenesis during cell division and interphase cortical ultrastructure to test the above hypothesis. I compare the developmental pattern as well as the ultrastructure of cirri, the dorsal bristle complex, membranelles, and general cytoplasm observed in Epiclintes, with that of other hypotrichs.

Materials and Methods

I isolated Epiclintes ambiguus from Great Bay Estuary near Adams Point in Durham, New Hampshire, USA (43°05'45" lat., 70°52'10" long.). I cultured populations of Epiclintes variously on either mass populations of diatoms scraped from estuarine substrates, then added to F_2 medium (GUILLARD and RYZHER, 1962) or on single species diatom populations of Bellerochea polymorpha or Phaeodactylum tricornutum in 25% seawater at 16°C.

Using light microscopy, I observed cells live as well as stained using a modified protargol technique. This technique and my procedure for processing cells for S.E.M. and T.E.M. are described in WICKLOW (1981a) with the following modification: to relax cells, several drops of 8% $MgCl_2$ were added to small volumes (approximately 25 ml) of culture medium in which cells were concentrated. I viewed cells using JEOL 100s T.E.M. or an AMR 1000 S.E.M.

All references to the cell will be relative to the cell's left or right; in ventral aspect the cell's left corresponds to the reader's right.

Results

Behavior and Ecology

Epiclintes ambiguus is an elongate, highly contractile and supple, epibenthic hypotrich. It is ubiquitous in most marine habitats including estuaries, sandy beaches, salt marshes, and tide pools. It feeds on many kinds of diatoms by gliding along the substrate sinuously while maneuvering its supple, slightly cephalized anterior end. It sometimes backs erratically to change direction; at times, under severe stimuli such as a change in osmolarity, it can back up rapidly for a distance of several millimeters.

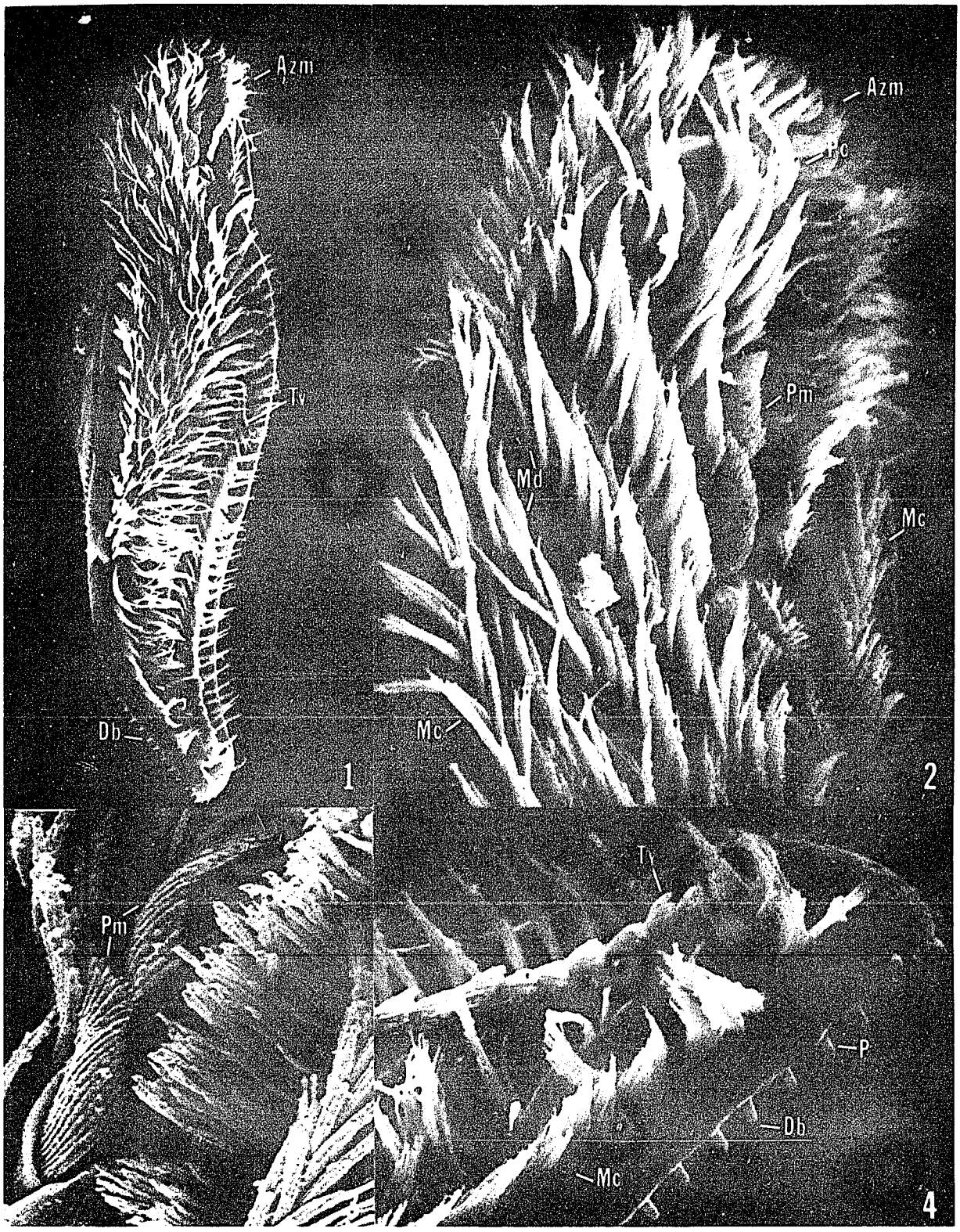
General Morphology

Epiclintes is generally narrow, elongate and slightly dorso-ventrally compressed. When relaxed it is 350-370 μm long, approximately 35 μm wide at the anterior end, approximately 50 μm at the midregion, and approximately 25 μm at the posterior end; when actively feeding, cell length increases considerably accompanied by a general narrowing in width. At these times cephalization of the anterior end is more prominent while the posterior end is attenuate.

The adoral zone of approximately 60 membranelles curves right, then posteriorly; all membranelles are positioned ventrally (figs. 1,2). A paroral membrane lies above the right buccal overture while the endoral membrane lies within the buccal cavity (figs. 2, 3).

Two paroral cirri are located at the anterior left ventral surface (fig. 2). Thirteen oblique cirral rows occupy the ventral surface between right and left marginal cirri; because these oblique

Fig. 1, 2, 3, 4. Sacnning electron micrographs of Epliclintes ambiguus (ventral aspect). The adoral zone of membranelles (Azm) curves around the anterior of the cell and is directed ventrally (figs. 1, 2); 13 oblique rows of median cirri (Md) lie between single left and right marginal cirral rows (Mc); a row of 29 transverse cirri (Tv) are located along the left posterior half of the cell (figs. 1, 2). The paroral membrane (Pm) lies within a cortical furrow above the right buccal overture (fig. 3); 2 paroral cirri (Pc) extend from the anterior, left ventral surface (fig. 2). Dorsal bristles (Db) emerge from cylindrical papillae (P) (figs. 1, 4). (X 920, 4000, 43000, 2900).


Azm
Tv
Db
1
Azm
iC
Pm
Md
Mc
Mc
2
Pm
Tv
P
Db
Mc
4

rows arise variously from frontal, somatic, or caudal primordia (discussed below) I shall refer to them generally as median cirri. The number of cirri per median row ranges from 5 to 22; median cirral rows in the midregion of the cell are generally longer. A group of approximately 29 transverse cirri subtend the median cirral rows on the left side of the cell (fig. 1).

On the dorsal surface are 3 rows of cylindrical papillae; within each papilla is a dorsal bristle that protrudes from an opening at the distal end of the papilla (fig. 4). Ventral to each papilla lie the bristle kinetosomes: the anterior bears the cilium, the posterior is a condylocilium.

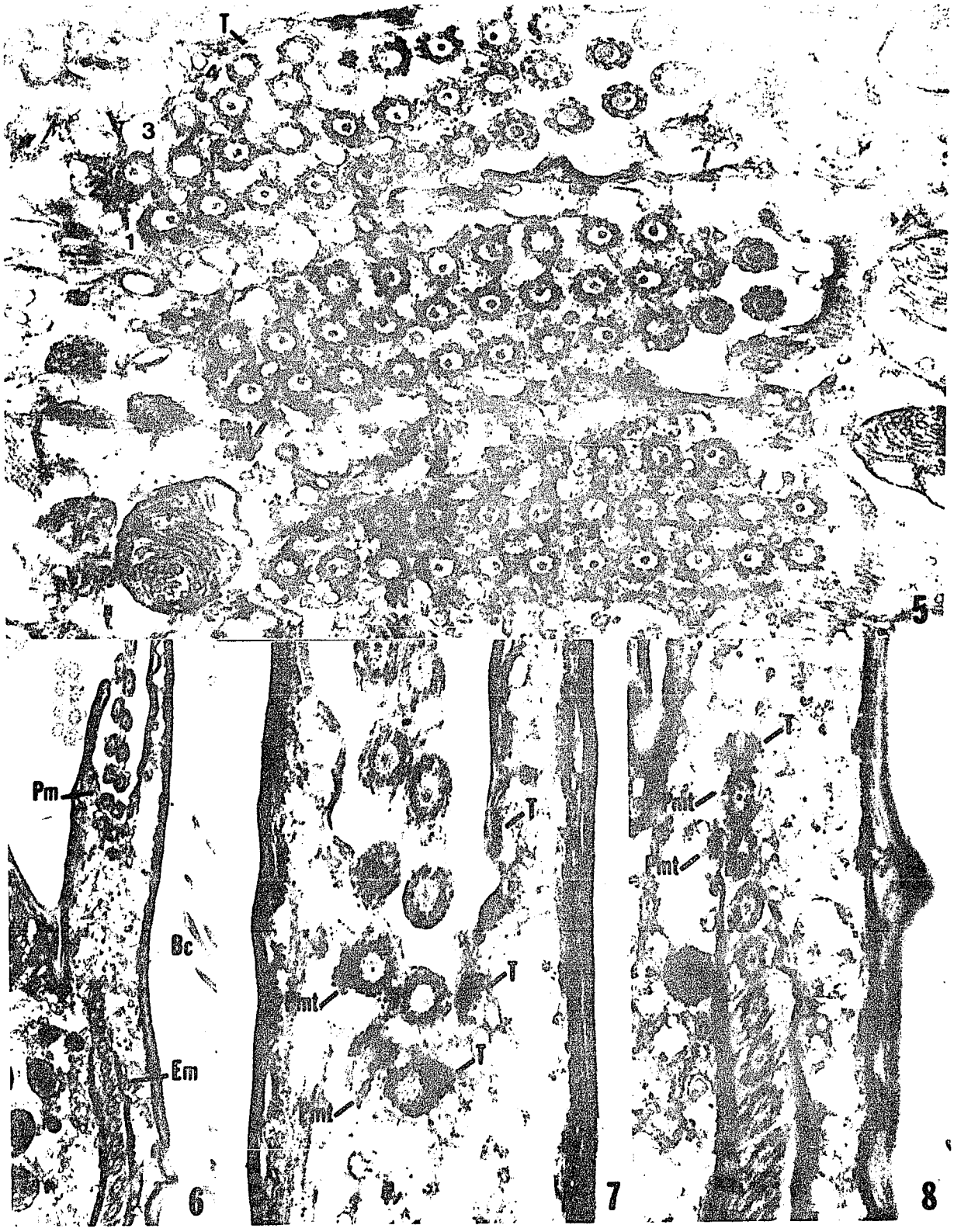
Ultrastructure

Buccal apparatus. Membranelles are paramembranelles, each consisting of 4 rows of kinetosomes. The first (posteriormost) and second rows are the longest rows and are of equal length (approximately 11 kinetosomes); the third row is shorter (by 2 kinetosomes) and the fourth row is shortest consisting of only 2 kinetosomes. Postciliary microtubules arise from row 1 kinetosomes; transverse microtubules arise from row 4 kinetosomes and those kinetosomes of row 3 not bordered by row 4 kinetosomes (fig. 5).

The paroral membrane consists of a longitudinal series of kinetosomal pairs (polystichomonade); it lies within a cortical furrow along the right buccal overture (figs. 3, 6, 7). Postciliary microtubules originate on the right side of the membrane then extend posteriorly whereas transverse microtubules arise on the membrane's left side and are directed toward the pellicle (fig. 7).

The single kinetosome row composing the endoral membrane

Fig. 5, 6, 7, 8. Transmission electron micrographs of the buccal apparatus. Membranelles comprise 4 rows of kinetosomes (numbered 1-4): the first 2 are longest and of equal length, the third is slightly shorter, the fourth is shortest. Transverse microtubules (T) originate from row 4 kinetosomes and those kinetosomes of row 3 not bordered by row 4 kinetosomes, postciliary microtubules (Pmt) arise from row 1 kinetosomes (fig. 5). The paroral membrane (Pm) consists of a row of kinetosomal pairs (polystichomonade) with cilia directed ventrally while the endoral membrane (Em) is a row of single kinetosomes (stichomonade) with cilia directed dorsally (fig. 6). Transverse microtubules (T) arise from the left, postciliary microtubules (Pmt) arise from the right of both oral membranes (figs. 7, 8). (X 28 500, 8 500, 33 100, 21 250)


T
4
3
1
5
Pm
Bc
Em
6
T
mt
7
T
mt
8

(stichomonade) lies within the buccal cavity; its cilia are directed medially (fig. 6, 8). Although rotated 180° relative to the membrane's long axis, the endoral kinetosomes possess postciliary microtubules along the right side and transverse microtubules along the left side of the row (as in the paroral membrane). This apparent parodox is explained in JERKA-DZIADOSZ (1981a).

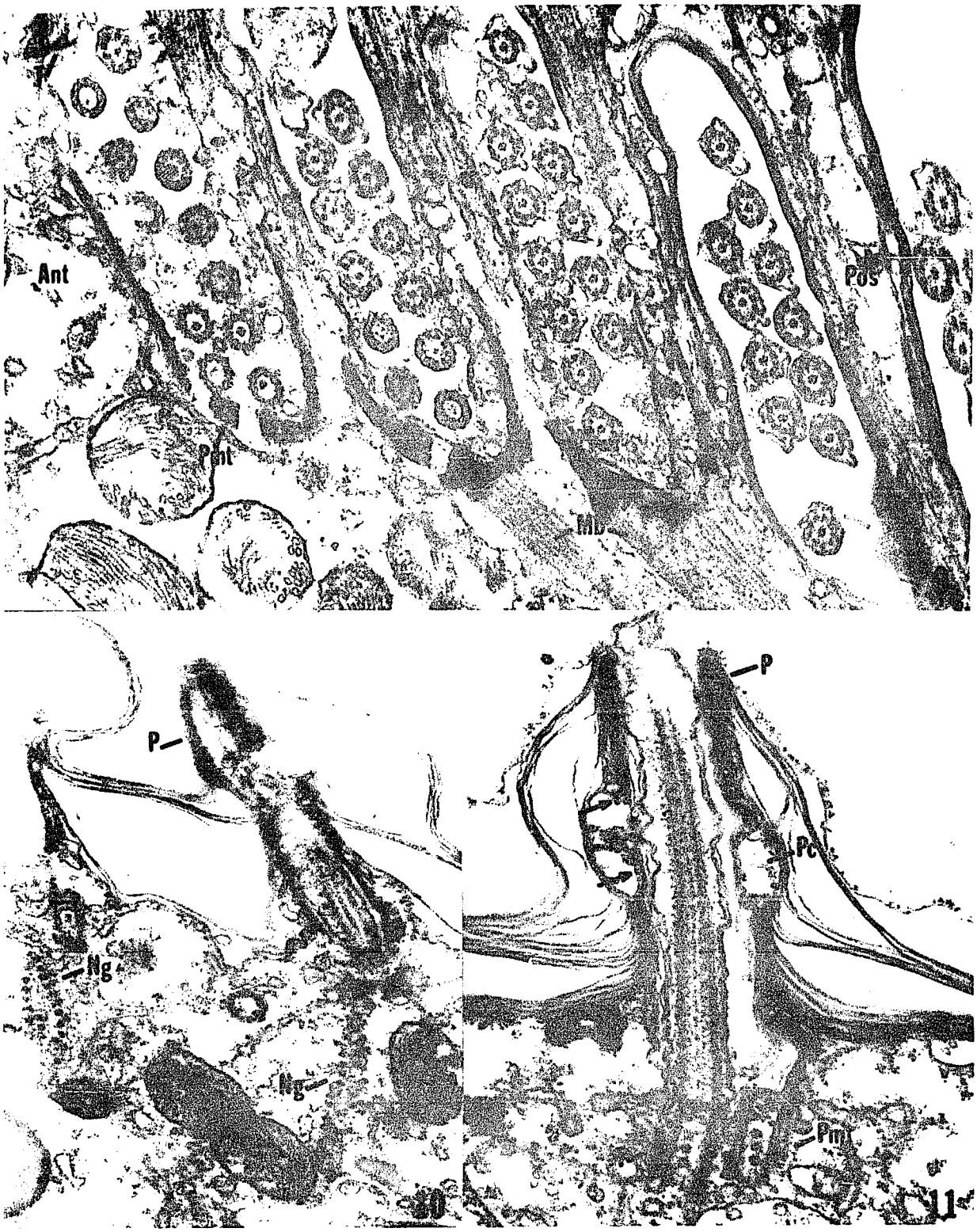
Cirri. All median and marginal cirral bases of Epiclintes are parallelogram arranged sets of kinetosomes: 2 kinetosomes wide and 6-8 kinetosomes long (fig. 9). Each cirrus lies in a cortical indentation at an approximately 60° angle to the long axis of the cell. Transverse cirri each consist of 5 rows of 8-10 kinetosomes. A fibrillar sheath surrounds each cirrus base distally, then descends to encircle the cirrus proximally as the peripheral cirral matrix; microtubular bundles radiate into the cytoplasm from this matrix. The largest of these (directed posteriorly from the right side of the cirrus) overlaps with the same bundle of the adjacent cirrus, thereby forming a continuous overlapping series visible at the right microscope level (fig. 9).

Postciliary microtubules arise from the posteriormost kinetosome of each cirrus and extend posteriorly to contribute to the posterior microtubular bundle. Transverse microtubules originate near the anteriormost cirral kinetosomes and extend directly toward the pellicle (fig. 9).

Dorsal bristles. Each dorsal bristle complex is a dikinetid with the anterior kinetosome ciliated, the posterior kinetosome nonciliated (a condylocilium). Dorsal cilia lie within cortical papillae that extend 1 μm from the cell surface; only the distal 0.5 μm of each

Fig. 9. Transmission electron micrograph of median cirri: anterior (Ant) is at the left, posterior (Pos) is at the right of the micrograph. Transverse microtubules (T) arise from anteriormost right kinetosomes; postciliary microtubules (Pmt) arise from posteriormost, left kinetosomes. The posterior microtubular bundles (Mb) extend posteriorly in an overlapping series visible at the light microscope level. (X 25 900)

Fig. 10, 11. Sagittal sections through dorsal bristle complexes. Linear arrays of nematodesmal granules (Ng) lie beside nematodesmal microtubules (fig. 10). Each bristle complex is a dikinetid: the anterior kinetosome is ciliated, the posterior kinetosome is non-ciliated. Bristle cilia lie within cylindrical papillae (P). Papillary cisternae (Pc) encircle the midregion of each papilla; microtubules (arrows) are associated with these cisternae (fig. 11). Transverse microtubules (T) arise from the anterior kinetosome while postciliary microtubules (Pmt) arise from the posterior kinetosome. (X 18 800, 31 700)


Ant
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Ng
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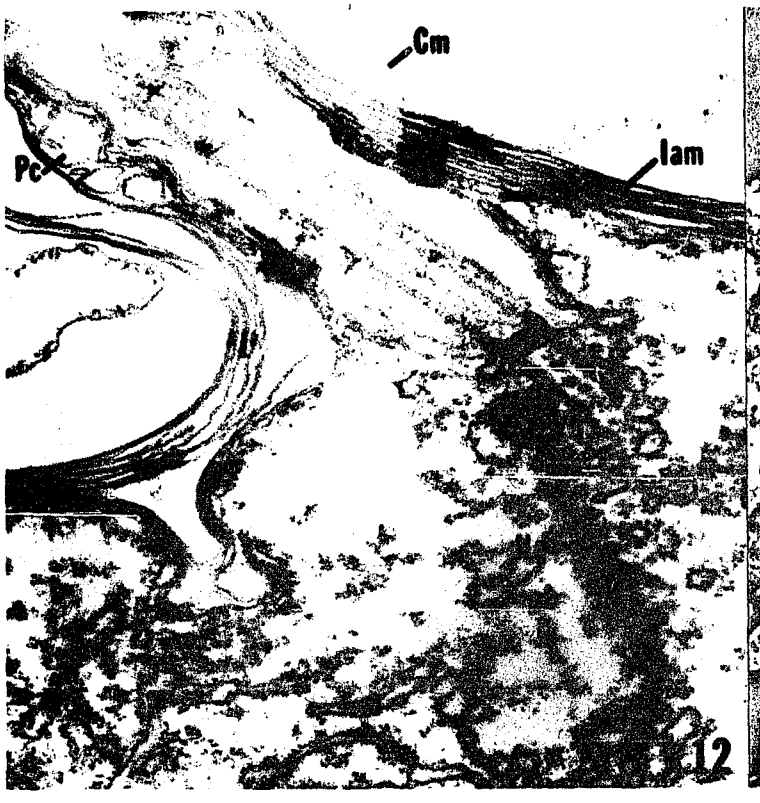
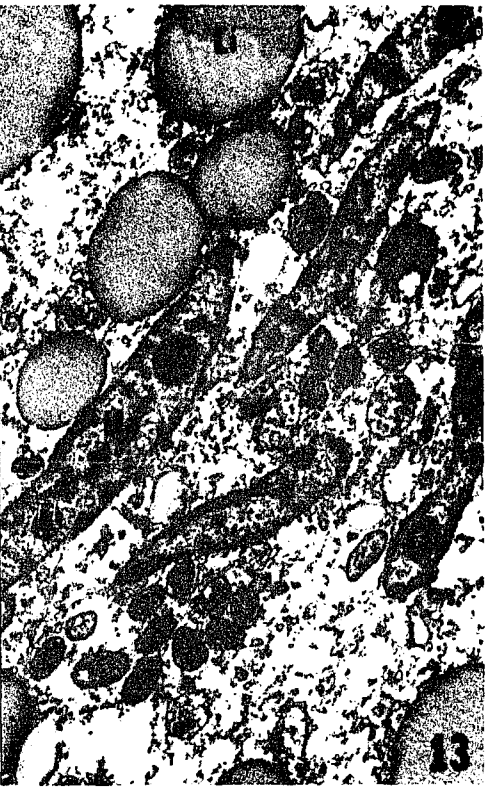
Pc
Pmt
11

Fig. 12. Sagittal section through a bristle complex showing the arrangement of nematodesmal granules along nematodesmal microtubules (Ne) which descend from bristle kinetosomes. Fine connections (arrows) link adjacent nematodesmal granules. The papillary cisternae (Pc) as well as the cell membrane (Cm) and a system of multiple membrane-like, intraalveolar materials (Iam) are evident. (X 33 000)

Fig. 13. Transmission electron micrograph showing the elongate, irregularly shaped macronuclei (Ma) and numerous lipid inclusions (Li). (X 6 000)


Cm
lam
Pc
12


13

cilium protrudes from the papilla (figs. 4, 10, 11, 12). Membrane bound vesicles, papillary cisternae, lie within and encircle the midregion of each cylindrical papilla. Microtubules are associated with these vesicles (figs. 11, 12).

Transverse microtubules arise near the anterior bristle kinetosome, postciliary microtubules arise from the posterior bristle kinetosomes (fig. 11). Nematodesmal microtubules descend into the cytoplasm from fibrillar material at the base of the bristle kinetosomes. Electron dense granules (d = 55 nm) are arranged in a linear array at 30 nm intervals along the nematodesmal microtubules (figs. 10, 12); adjacent granules are linked by fine connections.

Cortex and general cytoplasm. Just proximal to the plasma membrane lies a multilayered, membrane-like system. The outermost and innermost membranes of this system may represent alveolar membranes; up to 24 additional membrane-like layers lie within this alveolus (fig. 12). The cytoplasm contains a large number of variously sized lipid inclusions. Numerous irregularly shaped, elongate macronuclei are present (fig. 13).

Morphogenesis

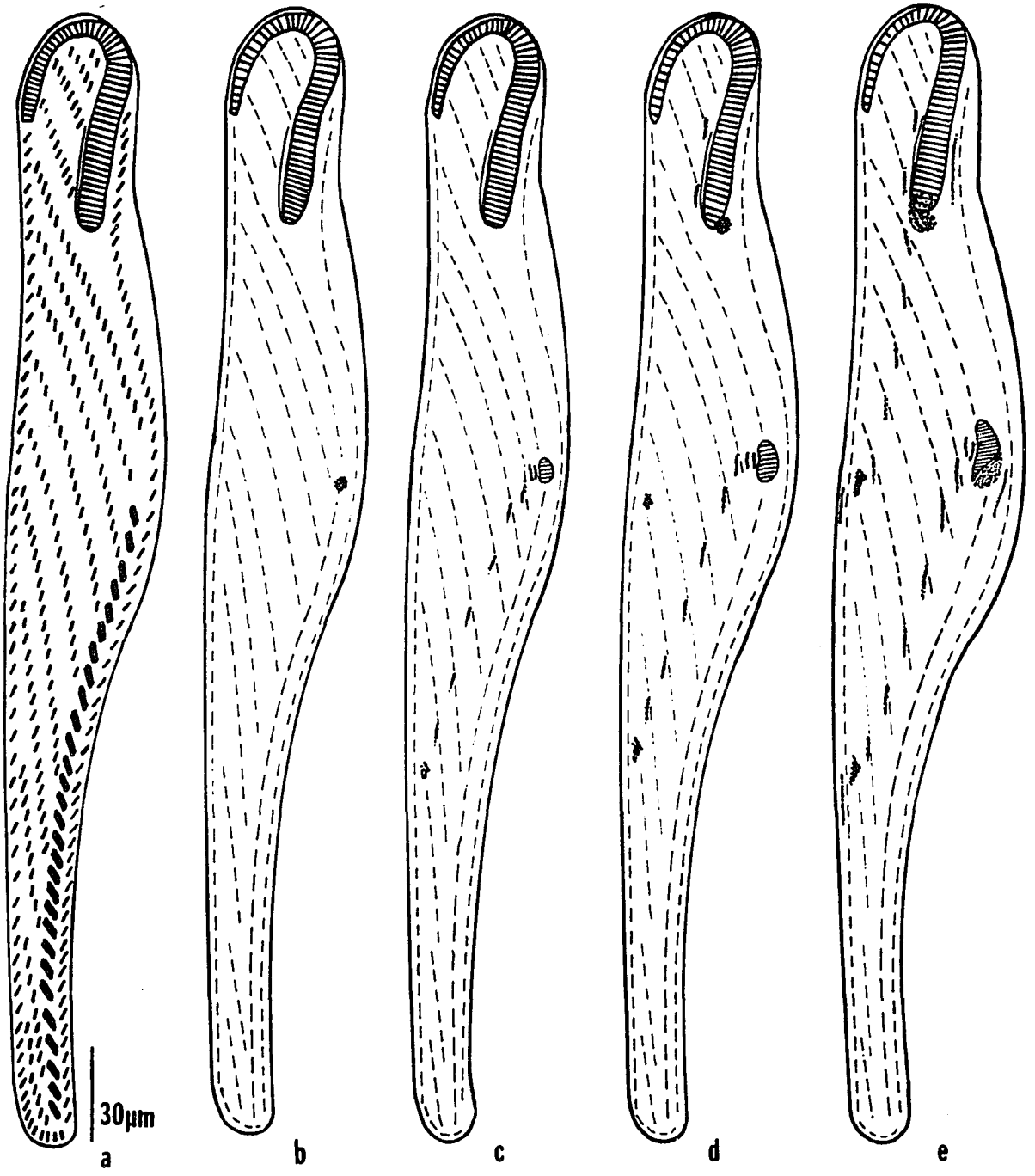
Cortical morphogenesis in Epiclintes occurs in 2 oblique developmental zones: an anterior zone of the future proter and a posterior zone of the future opisthe. The first cortical developmental event is the appearance of the opisthe oral primordium just anterior to the anteriormost transverse cirrus (figs. 14 a,b). This oral primordium (OP) begins as a dense mass of kinetosomes near the cell surface and soon begins differentiation into promembranelles; development of membranelles proceeds within a depression on the cell

surface in an anterior to posterior maturity gradient. The surface depression deepens posteriorly as new promembranelles are formed. Meanwhile, anterior promembranelles curve toward the cell's right forming the hooked membranellar zone characteristic of the interphase cell. During cytokinesis all membranelles emerge onto the cell surface (fig. 14 c-h).

As promembranelles begin to differentiate, 4 additional kinds of primordia appear on the ventral surface: frontal, paroral, somatic, and caudal (fig. 14 c). Single separate frontal and paroral primordia differentiate from the OP. Both paroral and endoral membranes as well as 2 paroral cirri differentiate from the anterior end of the paroral primordium. The opisthe frontal streak represents the sole frontal anlagen in both proter and opisthe; 5 frontal cirri differentiate from this streak (fig. 14 f,g).

Each ventral somatic primordium arises from kinetosomes of a median cirral row. In individuals with 13 median rows, six primordia are formed in this way in the opisthe (7 in the proter); each lengthens posteriorly. A caudal primordium appears as a mass of kinetosomes just left of the right marginal cirral row and anterior to the eleventh median row. This primordium differentiates into a longitudinal series of oblique streaks (fig. 14 e,f).

When the opisthe somatic primordia begin to lengthen, a proter OP appears as a cluster of kinetosomes just posterior to the parental membranelles (fig. 14 d). The subsequent differentiation and maturity of promembranelles from this primordium proceeds dorsally to the parental membranelles. The parental membranelles and paroral apparatus are dismantled and resorbed in a posterior to anterior direction; the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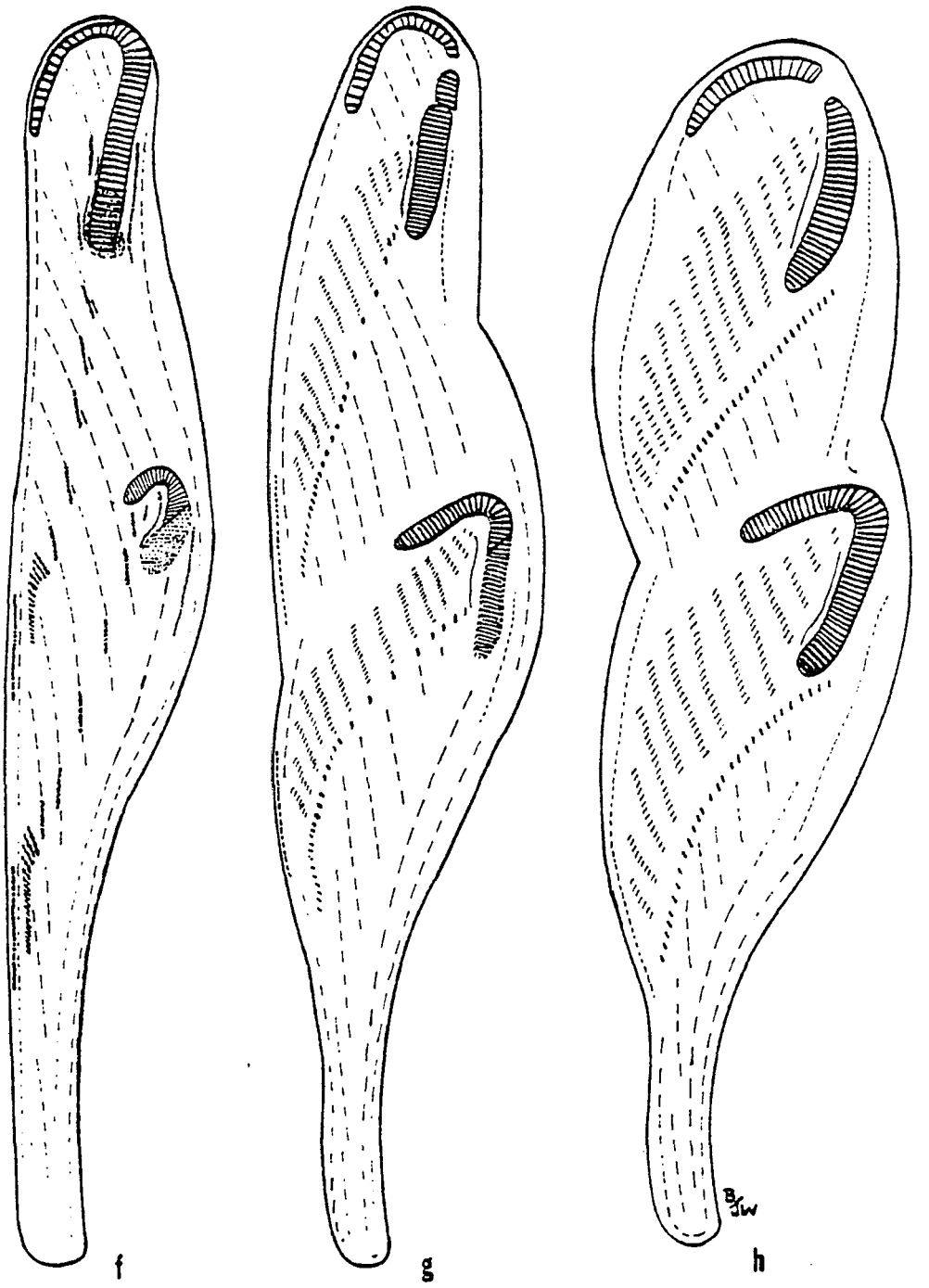

Fig. 14. Line drawings based on protargol stained specimens depicting a non-dividing cell (fig. 14 a) and a sequence of ventral, cortical morphogenetic stages during cell division (fig. 14 b-h). Black areas and lines represent ciliary organelles.

proter promembranelles erupt onto the cell surface just posterior to the retreating parental membranellar zone (fig. 14 e-h).

Frontal primordia are absent in the proter. Seven somatic primordia, however, arise from median cirral rows and a caudal primordium differentiates into a series of oblique streaks as in the opisthe. In both proter and opisthe the oblique streaks from each caudal primordium provide the posterior complement of median cirral rows; streaks from somatic primordia (along with the frontal streak in the opisthe) contribute the anterior complement of median rows (fig. 14 e-h). In this way 13 median cirral rows are formed in both the proter and the opisthe. The anterior 13 transverse cirri differentiate from the posterior end of each median cirral streak; the remaining (posterior) 16 transverse cirri arise independently from the caudal primordium of each daughter cell (fig. 14 f-h).

Marginal cirral primordia and dorsal bristle primordia arise by within row development during differentiation of the proter OP. Parental cirri are gradually dismantled and resorbed after cytokinesis is underway.

Discussion

Ultrastructure

In general, the buccal apparatus of Epiclintes is organized as in most other spirotrichs: membranelles comprising 4 kinetosomal rows (the first 2 longest, the third shorter, the fourth shortest) and a paroral apparatus consisting of outer stichomonade endoral membrane. The positions of microtubules associated with these structures resemble the general hypotrich pattern (see BAKOWSKA and JERKA-DZIADOSZ, 1978; GRIMES, 1972; PUYTORAC and GRAIN, 1976; WICKLOW, 1981a).

Cirri of Epiclintes also are organized as in other hypotrichs as well as some heterotrichs (se ALBARET and GRAIN, 1973; GRIM, 1972; GRIMES and L'HERNAULT, 1978; PUYTORAC et. al., 1976; WICKLOW, 1981a). The general agreement in arrangement of hypotrich cirral kinetosomes and associated microtubular ribbons reflect an orderly and conservative developmental process: early (primary) developmental events are more highly conserved than subsequent higher level developmental events (see below). Microtubular bundles associated with the peripheral cirral matrix differentiate late in development (JERKA-DZIADOSZ, 1980); these appear to be one of the most variable cirral constituents between hypotrichs (WICKLOW, 1981a).

Several interesting aspects of the cortical ultrastructure in Epiclintes appear unique. For example, the pellicle of Epiclintes comprises an outer plasma membrane subtended by a multilayered membrane-like system within alveolar membranes.

Other ciliates lack such a system within pellicular alveoli (for

review of ciliate membrane systems see ALLEN, 1978). Although most cliates possess alveoli in which electron dense intraalveolar material is absent, Euplotes (HAUSMANN and KAISER, 1979) and Certesia (WICKLOW, 1982b) both have alveoli that contain multilayered alveolar plates; other ciliates may possess disorganized material within alveoli (BÖHM and HAUSMANN, 1981).

BÖHM and HAUSMANN (1981) have determined the alveolar plates in Euplotes to consist mainly of protein with a fine coating of polysaccharide; these plates in Euplotes and Certesia apppear to contribute to cell rigidity. The intraalveolar layers in Epiclintes have a unique organization and must be supple to facilitate change in cell form in this highly contractile ciliate. Hence, the pellicular alveoli in Epiclintes appear non-homologous to alveoli of other ciliates studied thus far.

Although the general organization of the dorsal bristle complex of Epiclintes appears similar to other hypotrich dikinetids (see LYNN, 1981), certain anciliary features of the complex are unique. The tubular papillae from which bristle cilia emerge are absent in other hypotrichs. The dorsal surface of Aspidisca (as viewed with S.E.M, WICKLOW, unpublished) is studded with rows of globular papillae that presumably contain dorsal bristles; bristle cilia apparently do not project from the papillae. Hence, these papillae appear distinct from those in Epiclintes.

The nematodesmal granules that lie just ventral to bristle kinetosomes similarily are unique. These interconnected granules arranged in linear arrays, however, resemble the lasiosomes found between the ciliary membrane and axoneme of the bristle cilia in

Euplotes (RUFFOLO, 1976) and Certesia (WICKLOW, 1982b). The ultrastructural and functional organization of the dorsal bristle complex in Epiclintes, particularly the papillary cisternae, their associated microtubules, and the nematodesmal granules, need further study.

Morphogenesis

Four kinds of general ciliary primordia occur during morphogenesis in hypotrichs: frontal, oral, somatic, and ventral (WICKLOW, 1981). Most hypotrichs differentiate their entire ciliature from frontal, oral, and somatic primordia alone (see BORROR, 1979; BORROR and WICKLOW, 1982). In addition to these 3 kinds of primordia, Pseudourostyla differentiates a portion of its ventral median cirral rows from ventral primordia. Because of this divergent kind of primordium and its resulting aberrant developmental pattern, I separated Pseudourostyla from other urostylines at the superfamilial level (WICKLOW, 1981a). Epiclintes exhibits a fifth type of ciliary primordia: caudal primordia.

Frontal ciliature characteristic of hypotrichs is present in Epiclintes only as a single cirral row in the opisthe. In contrast to other hypotrichs, Epiclintes exploits caudal primordia as a means of forming a major portion of its ventral median cirri. During early development, caudal primordia appear similar to the ventral primordia of Pseudourostyla cristata (JERKA-DZIADOSZ, 1972; WICKLOW, 1981a); they differ, however, in the kinds of cirral rows produced (lateral, full-length longitudinal rows in P. cristata; oblique, posterior median rows in Epiclintes) and in production of transverse cirri (none in P. cristata and many in Epiclintes).

In an ultrastructural study of cirral morphogenesis in Paraurostyla

weissei, JERKA-DZIADOSZ (1980) showed the early development and differentiation of both frontal and marginal (somatic) cirral to be nearly identical. I expect caudal primordial development to proceed in a similar manner. Development of hypotrich cirri as well as other organellar complexes such as membranelles (GRIMES, 1972; JERKA-DZIADOSZ, 1981b; RUFFOLO, 1976) is based on the kinetosomal pair as an initial formative unit.

By extending the Structural Conservatism Hypothesis (LYNN, 1976) to morphogenetic processes, initial developmental events are expected to be highly conservative; more variation is expected as more complex differentiation ensue. Similiarities in higher level morphogenetic events such as developmental patterns (the pattern of growth and deployment of incipient organelles or organellar complexes) reflects recent common ancestry and can be used to demonstrate homologous structures. For example, I assigned Thigmokeronopsis jahodai to the Urostylina on the basis of its developmental pattern: midventral frontal cirri arise from a longitudinal series of oblique streaks (WICKLOW, 1981a).

Although sharing the possession of ventral primordia with Pseudourosytla, Epiclintes lacks the midventral ciliature characteristic of the Urostylina. The morphogenetic pattern of Epiclintes is divergent from other hypotrichs studied thus far.

Phylogenetic considerations. In 1972 BORROR provisionally included Epiclintes in the Urostylidae: he recognized E. ambiguus as the sole species of this genus, listing 7 synonomies. Later, in redefining the Urostylidae, BORROR (1979) excluded Epiclintes on the basis of absence of midventral cirri.

FAURÉ-FREMIET (1961) and TUFFRAU (1979) included Epiclintes in the family Keronidae. CORLISS (1979) also appended Epiclintes to the Keronidae but as incertae sedis, whereas JANKOWSKI (1979) place this genus in the family Oxytrichidae.

Classification of Epiclintes in so many different families is not surprising: its general anatomy and behavior, even at the light microscope level, display unique, even bizarre characters making taxonomic judgements difficult. The specific name chosen by MÜLLER in 1786 still seems justified.

Ultrastructural and morphogenetic data provide additional means to test the phylogenetic fit of Epiclintes to other hypotrich families. For example, the present study justifies exclusion of Epiclintes from the Urostylina: midventral cirri are absent (indeed frontal ciliature is limited to a single row of 5 cirri in the opisthe). When compared with Kerona and Oxytricha, Epiclintes exhibits significant ultrastructural and developmental differences. In Kerona (WICKLOW, 1979) most oblique cirral rows differentiate from frontal primordia while the remaining cirral rows arise from primordia which originate within parental rows in a similar manner as occurs in Paraurostyla weissei (GRIMES and L'HERNAULT, 1978). This information demonstrates the ventral ciliature of Epiclintes and that of Kerona to be non-homologous.

The pattern of cortical morphogenesis of Epiclintes is also quite different from that of Oxytricha (see GRIMES, 1972); this indicates a wide phylogenetic gap between these genera. Both Kerona and Oxytricha however, share a developmental pattern characteristic of sporadotrichine hypotrichs. The present study falsifies hypotheses that include

Epiclintes in current hypotrich suborders.

Clearly, Epiclintes possesses a unique combination of characters that distinguish it from other hypotrichs. Cortical morphogenesis is based on 4 kinds of primordia; median cirral rows arise from frontal (in the opisthe only), somatic, and caudal primordia. Stomatogenesis in the proter proceeds dorsal to the parental buccal membranelles, which are subsequently dismantled and resorbed. Initiation of the opisthe oral primordium occurs near the cell surface; further development then proceeds within a subsurface pouch that lengthens posteriorly. This combination of developmental features are absent in other spirotrichs studied thus far.

Specializations of the dorsal bristle complex - cylindrical papillae that house the bristle cilia, papillary cisternae, nematodesmal granules - as well as the multilayered, intraalveolar, membrane-like system of the cortex serve to render Epiclintes as unique among the hypotrichs. Homology of these structures with those of other hypotrichs is wanting.

In addition to ultrastructural and developmental anomolies, Epiclintes is also marked by its extreme degree of contractility. Although present in such forms as Psammomitra and Stichotricha, high contractility is uncommon in hypotrichs; contractility is commonplace in heterotrich and karyorelictid ciliates.

The organization of membranelles and cirri of Epiclintes is consistent with the general structural pattern within the Spirotrichida; the pattern of morphogenesis, paucity of a frontal ciliature, as well as ultrastructural characteristics of the cortex and dorsal bristle complex, however, are inconsistent with presently known spirotrich

patterns. These specializations suggest that Epiclintes has diverged from other hypotrich or heterotrich orders early in spirotrich evolution. This divergence, marked by behavioral, morphogenetic, and ultrastructural attributes, suggests a phylogenetic gap sufficiently wide to warrant separation of this genus into its own suborder: the Epiclintina.

Diagnosis of the Epiclintina

Order HYPOTRICHIDA STEIN 1859

Suborder Epiclintina (n. subord.)

Diagnosis. Highly contractile, elongate, multimacronucleate, marine epibenthic ciliates with slight anterior cephalization and extreme posterior attenuation. Ventral ciliature comprises numerous oblique rows of median cirri, the majority of which differentiate during morphogenesis from somatic and caudal primordia; frontal primordia are generally lacking except for a single frontal primordium producing one median row in the opisthe. Dorsal bristles project from cylindrical papillae. A system of multiple, membrane-like intraalveolar material is present within the cortex.

Family Epiclintidae (n.fam.)

Diagnosis. As above.

One genus; Type - Epiclintes STEIN, 1862

E. ambiguus (MÜLLER, 1786) BÜTSCHLI, 1889 (type by subsequent designation)

E. candatus BULLINGTON, 1940 (incertae sedis)

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